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HYPERPARASITISM: A SPECIES OF *HEXAMITA* (PROTOZOA, MASTIGOPHORA) FOUND IN THE REPRODUCTIVE SYSTEMS OF *DERO-PRISTIS INFLATA* (TREMATODA) FROM MARINE EELS*

A. V. Hunninen and Ralph Wichterman

INTRODUCTION

While working on phases of the morphology and life cycle of the trematode, *D. inflata,* from the small intestine of the eel, *Anguilla chryspa,* small active protozoa were observed inside the eggs and uteri of the flatworms. Further study showed this protozoon to be a flagellate belonging to the genus *Hexamita* and limited to the reproductive systems of the trematode. Our preliminary note (Hunninen and Wichterman, 1936) appears to be the first account of a trematode infected with a flagellate protozoon.

HISTORICAL

Several cases of protozoan parasitism of helminths are reported in the literature. Bütschli (1878) found in abundance a spindle-shaped flagellate in the intestine of the free-living nematode, *Trilobus gracilis.* He found some freely detached and others united to one another in clusters by their posterior extremities. Kent (1880) described this flagellate giving it the new generic and specific name of *Leptomonas Bütschlii.* Chatton (1924) found a large leptomonad flagellate in the gut of an undetermined free-living marine nematode and Goodey and Triffitt (1927) observed and studied in detail a leptomonad from the intestine of a free-living nematode, *Diplogaster longicauda.* Further observations on the parasitism of *D. longicauda* were published by Triffitt (1928). Theiler and Farber (1932) and in a more complete account (1936) found trichomonads in the intestine of the oxyurid nematodes, *Aspicularis tetraptera* and *Syphacia obvelata* of white mice. Since they found

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no morphological difference between the parasitic flagellate and *Trichomonas muris,* which was abundant in the intestine of the mouse, they concluded the trichomonads to be identical. Becker (1933) reported the presence of two motile specimens of *Trichomonas muris* in an egg of the oxyurid, *Syphacia obvelata,* also parasitic in white mice. It should be mentioned that Graybill and Smith (1920) demonstrated that the protozoon, *Histomonas meleagridis,* is transmitted by embryonated eggs of the nematode, *Heterakis gallinae.* The disease caused by the parasite in the intestine of fowl being called "blackhead" or histomoniasis. Two reports have been made of *Giardia* from nematodes. Thomson (1925) found them in the gut of the bursate nematode, *Vianella viscaciae,* from two South American rodents, *Viscacia viscacia.* Graham (1935) observed *Giardia* in the intestine of strongyloid nematodes, *Cooperia oncophora* from a young bull. However, both Thomson and Graham were unable to find *Giardia* in the intestine of the hosts of the nematodes in the few animals examined. Kudo and Hetherington (1922) described a microsporidian parasite of the spirurid nematode, *Protospirura muris,* of the common house mouse and Martin (1936) found larval trematodes (*Succinea* sp.) harboring a sporozoan parasite. According to him, the sporozoon was observed in both snail and trematode tissue, being present in three species of cercariae. In some cercariae, the infection was so great as to suggest interference with the completion of the life cycle of the trematode.

MATERIALS AND METHODS

The eels were collected daily at the Marine Biological Laboratory, Woods Hole, Massachusetts, during the months of July and August, 1936. For the most part, they were examined immediately after being brought into the laboratory or else placed in aquaria with running sea water until autopsied. They were killed by severing the head from the body; then the entire alimentary tract was quickly removed and cut into segments. The mucus and intestinal contents were carefully scraped and teased, then examined microscopically.

Observations were made on both living flagellates and fixed and stained slides. For the permanent preparations, infected trematodes were teased apart with needles on a cover-slip and immediately fixed in Bouin's, Hollande's or Schaudinn's fluid heated to 45° C after adding 5 per cent of glacial acetic acid. For staining, best results were obtained using Heidenhain's iron-alum-haematoxylin. Other infected trematodes were imbedded in paraffin and sectioned.

DESCRIPTION

The protozoon living in the body of the trematode belongs to the genus *Hexamita.*

In the living condition, the flagellates appear as small pyriform bodies moving in the typical hexamitid manner (Figs. 1 and 5). The long flagella are clearly discernible though the nuclei are observed with difficulty.

Greater detail can be seen in the fixed and stained preparations (Figs. 2, 3, and 4). Measurements of 60 individuals gave a range of length from 7.7 μ to 14.3 μ with an average of 10.2 μ and a range in width from 3.3 μ to 6.7 μ with an average of 5.5 μ. While some appeared to be broadly pyriform (Fig. 4) others were more slender (Fig. 3). In all cases, however, the protozoon is rounded anteriorly and pointed posteriorly.

Six flagella originate from the anterior end of the body, being about one and a half times the length of the organism and directed posteriorly. The two posterior flagella which are about twice the length of the body, continue from the axostyles.

Two compact club-shaped nuclei are located at the anterior end of the flagellate and extend slightly more than one-third of the body length. At times a clear definite nuclear membrane was observed about each (Fig. 4).

Generally, the cytoplasm is faintly granular and contains the more or less parallel axostyles.

LOCATION AND NUMBER OF HEXAMITA IN *Deropristis inflata*

An examination was made of 49 parasitized trematodes in order to find in what part of the reproductive organs the flagellates were most abundant. They were found commonly in the eggs, the uterus, the oviduct and the seminal receptacle. The flagellates occurred also in the vitelline glands of ten and in the testes of two of the 49 trematodes. Since this species of *Hexamita* is only about 11 μ in length and quite inactive in the dense tissues of the vitelline glands and testes as compared to the great activity in the other organs mentioned, it appears likely that the incidence in the vitellaria and testes may be higher than the data show, as some may have been overlooked in the earlier part of the work.

In many of the worms, the flagellates were present in large number, actively swimming or being carried to and fro by the peristaltic action of the uterus and seminal receptacle. Varying numbers were found in the eggs, some having a single specimen while in others 20 or more were counted. The amount of egg yolk destroyed or used up by *Hexamita* was seen to be directly proportional to the number of flagellates present in the eggs; those eggs with many *Hexamita* contained little or no yolk, while much yolk material was present in the eggs with few protozoa. Counts were made of 13 parasitized trematodes to determine the total number of eggs infected per worm. In five of these worms, *Hexamita* were found in all of the eggs (one worm having 300 eggs); in two worms 90 and 50 per cent were infected respectively, while in the others 14 per cent and less of the eggs contained the flagellates. It is easy to understand how such a large number of eggs can become infected because the seminal receptacle (which in some worms was literally filled with the flagellates) is a common site for these protozoa, which are thus in a suitable location to enter the eggs while they are in the process of formation.

NUMBER OF TREMATODES (*D. inflata*) PARASITIZED

Data were obtained on 124 trematodes from 35 eels to determine the total number of worms parasitized by *Hexamita* and to find out how many worms were infected in any single eel.

Hexamita were found in trematodes in 20 of the 35 eels; the other 15 harbored non-infected flukes. The worms in 14, 2, 2, and 2 of these eels were 100, 75, 50, and 33 per cent infected, respectively. In those eels where the worms were heavily infected with the protozoa, every one of the trematodes harbored the flagellates in large numbers. In the cases where the protozoa were present in some but not all of the worms, the infection rate was either very low (only a few per infected trematode) or in addition to the lightly-infected flukes, there were present some non-infected immature worms. Our data show that if in any one eel the infection of *Deropristis* with the *Hexamita* is heavy, the other trematodes present in that eel are also infected. We do not know at present whether the *Hexamita* are spread from worm to worm or whether the eels obtain the infection at some earlier stage in their life cycle and subsequently lose the infection while the worms retain it. These points will be solved only after we get additional knowledge on the life cycle of this flagellate and trematode.

DISCUSSION

The specificity observed in this case of hyperparasitism is very interesting. First, we have pointed out that the flagellate is limited to the reproductive systems of the trematode, chiefly the female organs. It was never found in the parenchyma, in the intestinal cecae, or in the excretory system, thus showing organ specificity. Secondly, as far as our present data show, we have some evidence of host specificity. The flagellates were not found in 23 *Zoogonus rubellus* or in 16 Hemiurids (Trematodes) that also live in the eel. The flagellates were also conspicuously absent in the intestinal contents of the eels; they occurred in very small numbers in only 2 of the 130 eels examined and in both these cases the fecal material contained heavily parasitized eggs from worms present in the intestine of the same eels. These protozoa, therefore, were most likely liberated from the infected eggs that were present. A total of 88 eels, free of *Deropristis inflata,* were examined and none of them harbored flagellates in the intestinal contents or in the mucosa.[1]

[1] Additional data collected during the summer of 1937 support the above finding. The intestinal contents and mucosa of 37 eels, harboring *Deropristis,* were examined and the *Hexamita* found in the fecal material of seven of these eels. The *Deropristis* in all seven eels were heavily infected with *Hexamita.* Seven of the other 30 eels harbored non-parasitized flukes. An examination was made of the intestinal contents for *Hexamita* of 28 eels that did not harbor the *Deropristis* and all were found negative. Thus far we have not found *Hexamita* in the intestinal contents of eels that are free from *Deropristis* or in eels with non-parasitized *Deropristis.*

Although it appears that the *Hexamita* which is parasitic in the trematode (*Deropristis inflata*) is not parasitic in the intestine of the eel, there is always the possibility that the eels which at the time they were examined were negative to the *Hexamita* may have harbored them at an earlier stage of their life cycle and later were freed of these protozoa. The trematodes, therefore, are still carrying the *Hexamita* which for any number of reasons have disappeared from the intestinal lumen of the eel. These points, however, need further study.

Of the thousands of parasitized eggs observed, the protoplasm was shown to be destroyed or used up by the protozoa in their metabolic activities, thus showing their true parasitic nature. While we found it generally true that an egg never develops once the parasite is inside it, we did observe a single instance where an egg with a living, developed miracidium also contained a single flagellate.

It appears very likely from the activity and number of flagellates in many of the eggs that multiplication of *Hexamita* takes place there.

The presence of *Hexamita* in only the reproductive systems of the trematode and its absence from other species in the eel as well as from its intestinal contents points toward an interesting life cycle for this protozoan parasite.

SUMMARY

1. A new species of *Hexamita* was found in the reproductive systems of the trematode, *Deropristis inflata*.

2. It ranged in length from 7.7 μ to 14.3 μ (average 10.2 μ) and in width from 3.3 μ to 6.7 μ (average 5.5 μ). The flagellate is pyriform with the anterior flagella about one and a half times the length of the body. The posterior flagella emanate from the axostyles and are about twice the body length. The two anteriorly-located nuclei are club-shaped and are slightly more than one-third the body length; cytoplasm homogeneous.

3. The flagellate was found in the reproductive organs of the host trematode, showing organ specificity. The present data indicates host specificity since the *Hexamita* were not found in other trematodes of the same eels or in the intestines of the eels.

4. Heavily parasitized eggs of the trematode do not develop into miracidia.

5. This appears to be the first report of a trematode being infected with a protozoon.

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EXPLANATION OF PLATE

All figures, except 1, drawn with the aid of a camera lucida. Figures 1, 2, 3, and 4 made at a magnification of 4000, figure 5 at 1540; all reduced one-half original size in printing.

FIG. 1. Typical shape of living specimen showing six anterior and two posterior flagella.

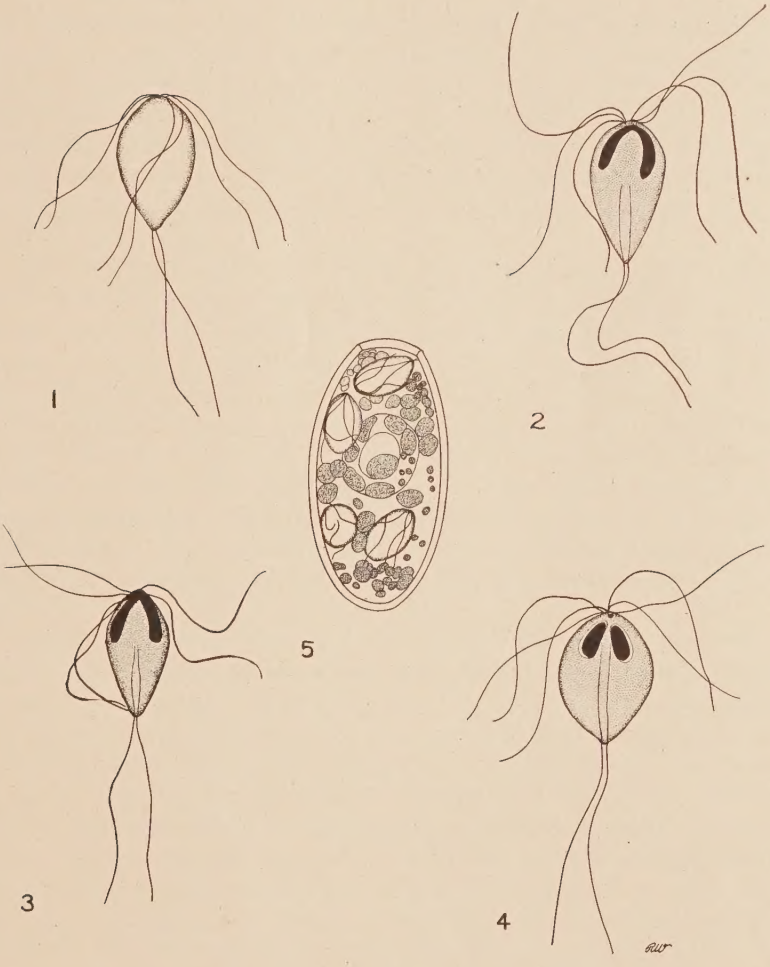
FIGS. 2, 3, and 4. Fixed and stained *Hexamita* showing flagella, nuclei, and axostyles.

FIG. 2. Typical individual. Schaudinn's fixative, Heidenhain's iron-alum haematoxylin.

FIG. 3. Typical individual. Hollande's fixative, Heidenhain's iron-alum haematoxylin.

FIG. 4. Broad specimen showing separated nuclei each one surrounded by a nuclear membrane. Schaudinn's fixative, iron-alum haematoxylin.

FIG. 5. Egg of a trematode as seen in the uterus containing four living *Hexamita* with yolk material and other inclusions.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SOME DIGENETIC TREMATODES FROM PUGET SOUND FISH[1]

LOWELL CLYDE LLOYD[2]

Knowledge of the parasitic fauna of the fish of Puget Sound, and in fact of the entire Pacific Coast of North America, is extremely meager. The works of such European investigators as Lühe, Looss, Odhner, Nicoll, and others have resulted in a fairly satisfactory knowledge of the digenetic trematodes of the fish of the European waters. In this country, Manter and Linton have described many species from the Atlantic Coast, works which apparently are both extensive and adequate on the trematodes of marine fish.

This study represents a beginning of a comprehensive investigation of the trematode fauna of the marine fish of Puget Sound. As yet, comparatively few of the possible host fish have been examined. The several species of salmon are especially worthy of more intensive study. Only the "humpback salmon," *Oncorhynchus gorbuscha* (Walbaum), and the "spring salmon," *O. tschawytscha* (Walbaum), have been examined in at all satisfactory numbers. This has shown an interesting similarity with the parasitic fauna (Odhner, 1905) of *Salmo salar* Linnaeus, the Atlantic species of salmon, as both *Brachyphallus crenatus* (Rudolphi 1802) Odhner 1905 and *Hemiurus levinseni* (Odhner) found in the stomach of *O. tschawytscha* (Walbaum) have been reported from *Salmo salar*.

Another interesting problem is the possible difference in the trematode fauna of certain littoral fish common to the San Juan and Puget Sound regions proper. Two host fish, *Ophiodon elongatus* (Girard) and *Leptocottus armatus* (Girard) have been examined in large numbers at Friday Harbor and a few from Seattle. At Friday Harbor *Ophiodon elongatus* (Girard) was found to be invariably parasitized by *Lecithochirium exodicum* McFarlane but the fish taken at Seattle were infested with *Genolinea robusta*. At Friday Harbor the common stomach parasite of *Leptocottus armatus* (Girard) was *Tubulovesicula* sp. (the subject of a separate study) while at Seattle this parasite was replaced by *Genolinea manteri*. Further study is, of course, necessary to determine how great a difference exists between the two regions.

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[1] Contribution from the Departments of Zoology and Oceanography of the University of Washington. This is the seventh of a series of publications on the parasites of Puget Sound fishes by John E. Guberlet and his students.

[2] Died, October 7, 1936. The manuscript was prepared for publication by John E. Guberlet and Harriet Exline Lloyd.

The author desires to acknowledge his indebtedness to Dr. T. G. Thompson, director of the University of Washington Oceanographic Laboratories, for courtesies and facilities extended during the course of this work. Grateful acknowledgement is made to Professor John E. Guberlet, University of Washington Department of Zoology, under whose direction this work has been carried on and for the use of his collections and library.

FAMILY MONORCHIDAE

Subfamily Proctotreminae

Telolecithus pugetensis Lloyd and Guberlet 1932

This species from the intestine of *Cymatogaster aggregatus* Gibbons has already been fully described elsewhere (Lloyd and Guberlet, 1932). Of considerable interest, however, was a marked variation in the incidence of infection which had taken place since the publication of the original description.

During the summers of 1930 and 1931, nearly all of the fish examined furnished from several to many worms. An uninfected fish was a rarity. In 1934, however, not a single specimen of *T. pugetensis* could be found although several hundred fish were examined from localities in the San Juan Islands where, in previous years, parasitized fish had been secured. Again, during the summer of 1935, several hundred fish were examined and in all, only 3 of the worms were secured.

A possible explanation for the absence of the parasties during 1934 and 1935 involves two factors, first, the semi-migratory habit of the host and second, the unusually warm winter of 1933–34. Eigenmann (1892: 412) said concerning the migration of *Cymatogaster aggregatus*: "During fall and winter this species is rarely seen as it then probably lives in deep water a short distance off shore or among fields of *Zostera*. In November and December it approaches the shore in large numbers and is caught with hook and line off the wharves in San Diego and San Francisco bays. The largest females become gravid about the first of December."

In Puget Sound the return of the fish to littoral regions may possibly be somewhat later. It is not likely that the littoral migration of *Cymatogaster* was much affected but it is probable that the seasonal abundance of the intermediate host was influenced. Thus, it is possible that, at the time of the return of the fish to littoral regions, the intermediate host of *T. pugetensis* had already reached and passed its maximum period of abundance and the parasite for this reason failed to reach its definitive host, at least in appreciable numbers.

FAMILY ALLOCREADIIDAE

Subfamily Allocreadiinae

Cymbephallus vulgaris Manter 1934

Figure 1

Two specimens of a trematode identical with, or closely allied to, this species were secured from the intestine of an "eel pout," *Lycodopsis pacificus* (Collett). The worms were dead and somewhat macerated and many details of their anatomy obscure. The following brief description is based largely on the best preserved specimen.

Length 1.75 mm, greatest width 0.52 mm, preacetabular region 0.45 mm, postesticular, 0.45 mm. Oral sucker terminal, 0.15×0.15 mm, ventral sucker, 0.28×0.23 mm. Pharynx, 0.1 mm in length and esophagus over twice this length. Gonads are all lobed with greater diameter transverse. Ovary 4 (or 5) lobed, 0.16×0.07 mm, situated in front of anterior testis; anterior testis, 0.17×0.09 mm; posterior testis, 0.22×0.17. Eggs collapsed and measurements uncertain but at least 90 microns in length and normally would probably exceed 100 microns.

The principal respect in which these specimens seem to differ from Manter's description (1934) is in the relatively smaller ventral sucker. In Manter's specimens the ventral sucker approaches a size three times that of the oral sucker whereas here it is barely twice as large. However, the species is variable and apparently quite cosmopolitan, Manter reporting it from sixteen different hosts. In the absence of more favorable material, it is thought best to assign these specimens to Manter's species.

Subfamily Opecoelinae

Opecoelina theragrae n. sp.

Figures 2–6

Ozaki (1925) described the genus *Opecoelus* which he established as the type of the family OPECOELIDAE. This family was treated in greater detail by the same author in another paper (1928) and later (1929), he described three species of *Coitocaecum* and attempted to justify the erection of the families COITOCAECIDAE and OPECOELIDAE. The status of these families depends principally on the taxonomic importance attached to the presence of an anal opening in trematodes. Fuhrmann (1928) accepted Ozaki's (1925) conception of the family OPECOELIDAE, and included in it the genera *Opecoelus, Opecoeloides* and *Coitocaecum*. Two other genera *Opegaster* and *Anisoporus* were added to the family by Ozaki (1928) but *Coitocaecum* was removed and made the type of a new family.

Odhner (1928b) erected the genus *Opecoeloides* for *Distomum furcatum* Bremser and considered both *Opecoelus* and *Opecoeloides* as aberrant ALLOCREADIIDAE because of their great morphological similarity to

Podocotyle. Stunkard (1931) reviewed the subject of the occurrence of anal openings in trematodes and came to the conclusion that "the presence or absence of secondary connections between the alimentary tract and the exterior is not a character of great taxonomic importance in the digenetic trematodes." He therefore reduced the OPECOELIDAE to the status of a subfamily of the ALLOCREADIIDAE, and eliminated the family COITOCAECIDAE by placing *Coitocaecum* in the subfamily OPECOELINAE. Yamaguti (1934) recognized the two families, OPECOELIDAE and COITOCAECIDAE but Manter (1934) included, without comment, his genus *Opecoelina* in the subfamily OPECOELINAE. Considering the very close morphological agreement of *Opecoelus* and other members of the group with *Podocotyle* their removal from the ALLOCREADIIDAE does not seem to be justified and Stunkard's classification is, undoubtedly, to be preferred.

Manter (1934) erected the genus *Opecoelina* describing two species, *O. scorpaenae* and *O. helicoleni*. *Opecoelina* is distinguished from *Opecoelus* principally because of the presence of a seminal receptacle and the absence of the finger-like extensions of the ventral sucker which are present in *Opecoelus*. In the present material there are no extensions of the sucker proper although sagittal sections show somewhat similar appearing extensions of the body wall. These are not apparent in medial sagittal sections but show in more lateral sections (fig. 5). Manter's two species are similar and the present material does not differ greatly from either of them, but in many respects seems to be intermediate.

The worms were not observed while alive and this description is therefore based entirely on whole mounts and sections.

Body form elongate and flattened, tapering slightly at either extremity, widened at ventral sucker and slightly constricted behind it, also frequently constricted opposite testes. Length 3.0–4.0 mm; greatest width about 0.5 mm; preacetabular region 1/7–1/10 of body length and post-testicular about 1/5. Oral sucker terminal averaging 0.17 mm in diameter, ventral sucker appreciably less than twice this; prepharynx short; esophagus about twice length of pharynx and extends to anterior border of ventral sucker; ceca extend to near posterior end where they join and open to exterior by short, common canal; anus dorsal, in slight depression, just posterior to excretory pore; excretory vesicle extends anteriorly to level of ovary.

Testes elongate oval 0.33–0.40 × 0.21–0.26 mm, one behind the other just posterior to mid-body; ovary four-lobed, oviduct arising from anterior lobe; seminal receptacle and yolk reservoir dorsal and to the left of ovary, Laurer's canal present; yolk glands broken opposite testes and coming together between and behind them; cirrus pouch thin-walled, reaching posteriorly half way to ovary and enclosing prostate and distal portions of seminal vesicle; genital pore to left just behind pharyngeal level; eggs 88–92 × 46–50 microns.

Host.—*Theragra fucensis* (Jordan and Gilbert), Puget Sound pollack.

Location.—Intestine.

Locality.—Seattle, Washington.

Type.—U.S.N.M. No. 9077.

A peculiarity which appears to be generic since it is mentioned by Manter in *Opecoelina scorpaenae* is the occasional atrophy or absence of one of the testes. Several specimens show a decided body constriction opposite the testes with more or less atrophy of one or both of them. In one case the posterior testis is entirely absent and the region occupied by a stellate scar-like area. Likewise the long cirrus frequently inserted into the metraterm of the same individual has been noted by Manter in *O. scorpaenae* and is characteristic of *O. theragrae.*

Opecoelina theragrae is similar to *O. helicoleni* Manter in general body form and in many details of anatomy but it differs in the following respects: ventral sucker more anterior and less than twice the size of oral sucker, post-testicular region relatively shorter, seminal vesicle extends ½ way to ovary as compared to ⅓ in *O. helicoleni,* testes are elongate oval rather than rounded, and the genital pore is apparently slightly more anterior. Eggs are larger, 88–92 × 46–50 microns, as compared to 72–74 × 41–42 microns.

The principal points of difference from *O. scorpaenae* Manter are as follows: larger, more elongate body; shorter preacetabular region; ventral sucker less than twice size of oral sucker; testes apparently never lobed and are separated by vitellaria; and eggs are larger (70–78 × 37–47 microns in *O. scorpaenae*).

As will be apparent, many of the differences might readily be due to normal variation in such contractile forms as the trematodes. Differences in egg size and sucker ratio are significant between *O. theragrae* and *O. helicoleni.* Egg size, sucker ratio and the extension of the vitellaria between the testes separate *O. scorpaenae* from the above species.

FAMILY AZYGIIDAE

Otodistomum veliporum (Creplin 1842)

This species has been found quite constantly in the stomach of the "barndoor skate," *Raja binoculata* Girard. It has been reported by Manter (1926) from this host at Friday Harbor and has received adequate morphological treatment by him in connection with his detailed study of *Otodistomum cestoides* (Van Beneden).

FAMILY HEMIURIDAE

Looss (1907) treated the morphology of this family in detail and established criteria for the determination of genera and species. He lists (p. 84) the following characters as of little value for this purpose: relative size relationships of the two parts of the body; relative extent of intestinal ceca and coils of uterus; relative position of organs in "Hinterkorper," i.e., the post-acetabular portion of the body proper.

Characters considered as important for the determination of genera and species are given by Looss (p. 97) in order of their importance as follows: (a) structure of genital organs particularly of the terminal genital ducts; (b) form of excretory vesicle; and (c) nature of cuticle and suckers, as well as the general body form and presence or absence of the caudal appendage.

Various terms have been employed for the major body regions in the HEMIURIDAE. Looss used the terms "soma" for the body proper, "abdomen" for the caudal appendage, "Vorderkorper" and "Hinterkorper" for pre- and post-acetabular portions of the soma. Nicoll (1915) followed Looss in applying the term "soma" to the body of the worm but used "ecsoma" or "appendix" for the caudal appendage. Manter (1926) used the terms "tail" or "tail appendage" for that structure. Here the following terminology is used: *soma* for the body proper, *ecsoma* for the caudal appendage and *pre-* and *post-soma* for the pre- and post-acetabular regions of the soma.

Several theories in regard to the origin and function of the ecsoma in the HEMIURIDAE have been advanced. Early authors were of the opinion that the ecsoma was a modified cercarial tail but Wagener (1860) noted that the tail appendage of the appendiculate distomes was in no way related to this structure. Later, Monticelli (1891: 516) opposed this theory and considered the ecsoma to be a persistent and structurally modified cercarial tail but Looss (1896: 134) attempted to show that the grounds cited by Monticelli in favor of this conception were not valid. Pratt (1898) regarded the "tail" of the hemiurids as nothing more than a peculiarly modified part of the excretory bladder. Looss (1907: 73) agreed with this view and presented additional observations in support of this idea of the origin of the ecsoma.

In regard to the function of the ecsoma Looss noted that it occurs primarily in stomach inhabiting forms and not in *Aponurus* or *Aphanurus* which inhabit the esophagus nor in *Lecithaster* which is found in the intestine. Furthermore it occurs in the larger hemiurids, not in the above mentioned small ones, and is largest and best developed in the largest forms.

Looss suggested that the thick cuticle necessary to resist the action of the gastric juice is too thick to permit of the functions which it probably normally performs in trematodes, i.e., absorption of food and possibly also respiration. The ecsoma is, according to Looss, provided with a thinner cuticle but can be retracted and protected at times when gastric acidity and enzyme concentration is high.

A study of the several species of HEMIURIDAE included in this paper has not shown the cuticle of the ecsoma to be appreciably thinner than that of the soma. However, the cuticle of the two regions does stain

quite differently. The cuticle of the ecsoma stains sharply and brilliantly with the cytoplasmic stain, while that of the soma is stained very slightly and not sharply. Probably this means nothing more than that the cuticle of the soma has been exposed to adverse environmental influences to a greater extent than has the cuticle of the ecsoma. It is possible that it indicates an intrinsic difference in the chemical or physical properties of the cuticle covering these two body regions.

That the state of extension or retraction of the ecsoma greatly affects the form and topography of the organs lying in the post-soma was noted by Looss (1907: 80) and therefore such relationships of organs in this part of the body are of little value for taxonomic purposes. In contrast to the variable post-soma, however, the pre-soma of hemiurids "shows a quite stable character, so stable in fact that a well preserved pre-soma with the acetabulum is practically sufficient for the identification of species."

The nature of the terminal genital ducts and "cirrus pouch" was emphasized by Looss (p. 98) in his key to the genera of HEMIURIDAE. Manter (1926) discussed the appropriateness of the term "cirrus pouch" in connection with hemiurids and suggested the use of "sinus sac" in its place. Looss was aware of the fact that the term "cirrus pouch" was not strictly applicable to hemiurids but said (1896: 127) that he used it for the sake of simplicity. It seems to the author that morphological terms should wherever possible have definite and restricted meanings and that Manter is justified in his use of the term "sinus sac" for the muscular pouch surrounding the genital sinus or ductus hermaphroditus. The term "cirrus pouch" may then be reserved for the similar structure limited to the male genital duct and frequently enclosing, besides the ductus ejaculatorius, the pars prostatica and seminal vesicle.

Looss used the term "true cirrus pouch" to mean a sinus sac forming a continuous muscular sheath or pouch around the genital sinus. Here Manter's term "sinus sac" is employed and is called a *complete sinus sac* where it forms a continuous structure while in such forms as the STERRHURINAE where it consists of isolated muscle fibres the term *incomplete sinus sac* is used.

Another structure present in some hemiurids is the small sucker-like pit lying on the ventral surface a short distance in front of the acetabulum. It has been referred to as "ventral pit" and "cervical pit" and should not be confused with the general concavity frequently present on the ventral surface between the suckers and giving the anterior region of the worm a spoon-like appearance. For it, the term *pre-somatic pit* is used. This structure is of special interest in *Lecithochirium exodicum*.

In regard to the taxonomy of the family as a whole the principal question has been whether to consider it in a restricted sense, excluding

the genera placed by some authors in the families SYNCOELIIDAE and ACCACAELIIDAE, or whether to consider these groups as subfamilies of the HEMIURIDAE.

Poche (1925: 199) discussed this matter in detail and came to the conclusion that the SYNCOELIIDAE and ACCACAELIIDAE cannot logically be separated from the HEMIURIDAE to the extent of giving them equal family ranking with that group. He, therefore, considered these two groups as subfamilies of the HEMIURIDAE. Fuhrmann (1928), following Dollfus (1923) and Odhner (1928a), restricted the family HEMIURIDAE and accepted the family rank of SYNCOELIIDAE and ACCACAELIIDAE.

Neither Dollfus nor Odhner gave their reasons for raising the above mentioned groups to the status of independent families and Fuhrmann included in the family SYNCOELIIDAE such genera as *Genolinea* Manter and *Derogenoides* Nicoll which certainly belong with the HEMIURIDAE. Other members of this family, to be sure, depart more widely from the HEMIURIDAE. Of these the genus *Syncoelium* is the only one met with in the present study. The structure of this genus, however, is rather easily derived from the more typical hemiurid type. Modification of the terminal genital ducts toward the structure found in *Syncoelium* is seen in *Derogenes* and especially in *Progonus* (= *Genarches*). Splitting of testes and ovary into distinct follicles is a striking feature in *Syncoelium* but probably not of great significance. The vitellaria of *Syncoelium* are likewise divided into 6–8 distinct follicles with 7 the most common number (the 4+3 lobes of the HEMIURIDAE). Neglecting the follicular nature of the genital glands, their arrangement is similar to that found in the HEMIURIDAE and the course of the uterus is not dissimilar. A seminal receptacle is absent in *Syncoelium* but as noted by Lloyd and Guberlet (1936) is probably represented by a diverticulum of the oviduct enclosed by Mehlis' gland.

The ACCACAELIIDAE differ from typical HEMIURIDAE in somewhat the same respects as do the SYNCOELIIDAE. In addition the vitellaria of the ACCACAELIIDAE are tubular and the intestinal ceca open into the excretory vesicle. In regard to the second character the taxonomic importance of secondary connections of the intestinal tract to the exterior has already been discussed in connection with the OPECOELINAE and the conclusion reached that no great significance need be attached to this character. The nature of the vitellaria is quite different from anything found in HEMIURIDAE but hardly sufficient to be used as a family character.

The author is inclined to the view that, while there are decided differences between the groups under discussion, the undoubted similarities of the SYNCOELIIDAE and ACCACAELIIDAE to the HEMIURIDAE are such that they cannot be logically separated from the latter as separate families.

Poche's classification has therefore been followed and the SYNCOELIINAE and ACCACAELIINAE are considered subfamilies of the HEMIURIDAE.

As regards the morphological features which Looss (1907) considered of taxonomic importance this study has, on the whole, tended to confirm their validity. Disagreement must be expressed, however, with the general statement of Looss (1907: 96) that those forms with a complete sinus sac lack a metraterm. A short but definite metraterm is present in *Hemiurus levinseni* Odhner and the genera *Genolinea* Manter and *Tubulovesicula* Yamaguti have well developed metraterms and well developed, complete sinus sacs as well.

In seeking to find other morphological features which might prove of value for taxonomic purposes the nature of the parenchyma has seemed to offer possibilities. In the STERRHURINAE it is decidedly vesicular. The genus *Brachyphallus* is similar in this respect. In *Hemiurus levinseni,* the only member of the subfamily HEMIURINAE available for study the parenchyma is very small in amount and is not markedly vesicular. The genus *Genolinea* has a compact, fibrous appearing parenchyma and that of *Derogenes varicus* (O. F. Müller) is quite similar. However, the genus *Tubulovesicula* shows a decidedly vesicular parenchyma but cannot otherwise be considered as closely allied to the STERRHURINAE as the structure of the terminal genital ducts is entirely different. Further study of other genera will be necessary to determine what, if any, taxonomic importance can be assigned to this feature.

Subfamily Derogenetinae

Derogenes varicus (O. F. Müller 1784)

Figures 7–9

This species is considered as probably the most widely distributed trematode parasitizing marine fish. In all, over 50 hosts have been reported (Manter, 1934) as harboring it. Its occurrence in this region completes evidence of its circumpolar distribution.

Three hosts are recorded here as harboring this parasite. At Friday Harbor, it was taken from the "ling cod," *Ophiodon elongatus* (Girard) and the "rock cod," *Sebastodes maliger* (Jordan and Gilbert), while at Seattle it occurred in the "smooth sculpin," *Leptocottus armatus* Girard. It is not abundant as only a dozen specimens were secured from over 60 rock cod and a single specimen from some 25 ling cod. Over 100 sculpin were examined at Friday Harbor without meeting with this parasite while the single specimen collected from this host was found after examining 9 of these fish secured at Seattle.

Odhner (1905) gave a detailed account of the anatomy and synonomy of *Derogenes varicus.* Manter (1926) also gave a brief account of its

anatomy and figured an example from Maine. The following table will serve for comparison.

	Rock Cod	*Ling Cod and Sculpin*	*Odhner 1905*
Length*	1.46	1.9–2.0	1–3
Width	0.47	0.5–0.6	¼ Length
O.S.	0.17 × 0.16	0.23–0.25 × 0.28–0.32	0.17–0.23
V.S.	0.40 × 0.42	0.38–0.40 × 0.37–0.38	0.33–0.55
Pharynx	0.08	0.08	0.07–0.13
Testes	0.1 × 0.1	0.19–0.20 × 0.17–0.21	?
Ovary	0.23	0.21	?
Eggs	54 × 28	52–56 × 26–28	54–66 × 28–33

* All measurements in mm except eggs in microns.

The specimens from the ling cod and sculpin appear to depart slightly from the limits assigned by other authors particularly in the relatively larger oral sucker and larger testes, ovary and vitellaria. However, the differences are not great and material is not available to determine whether they are constant. They are, therefore, included in this species along with the more typical material from the rock cod.

For details of anatomy Odhner (1905) may be consulted. Mention should be made of one point of considerable importance in which Odhner's description does not agree with the results based on a study of the material from the rock cod. This is in regard to the presence of a sinus sac in this species.

Odhner said (1905: 361): "Diese Teile (the genital sinus) werden nun von einem kugeligen, ziemlich dünnwandigen Cirrusbeutel umschlossen." He figured a sinus sac (Cirrusbeutel) in a sagittal view of the anterior end of this species (Taf. IV, fig. 7). In every respect Odhner's description of the terminal genital ducts agrees with the present findings except that we fail to find any semblance of a complete sinus sac. A few isolated muscle fibres, relatively even fewer than in *Lecithochirium,* are all that could be considered as representing even an incomplete sinus sac (fig. 8). The living worm or whole mount sometimes appears to possess a sinus sac but study of sections fails to reveal its presence.

Genolinea Manter 1925

This genus is placed in the subfamily Derogenetinae by Manter (1934). It is doubtful that the genus properly belongs in this subfamily if Looss' taxonomic criteria are to be accepted, as the structure of the terminal genital apparatus is quite different in *Genolinea.*

Genolinea laticauda Manter 1925

Figure 10

This species has not been found in the present study but through the courtesy of Dr. H. W. Manter it has been possible to examine one of

his paratypes for comparison with two species of this genus taken from Puget Sound fish. The examination of the paratype of *G. laticauda* in the light of information gained from study of the local species has permitted slight additions to the description of the species as given by Manter (1925; 1926).

Manter did not mention a seminal receptacle but it is visible in his paratype as an elongate oval structure measuring 0.16 mm in length by 0.07 mm in width and situated dorsally and left to the ovary. MacFarlane (1936) recorded *G. laticauda* Manter from the stomach of the "marbled sculpin," *Scorpaenichthys marmoratus* (Ayres) at Departure Bay, British Columbia. He did not refer to the seminal receptacle but his figure shows an organ which resembles that structure. A seminal receptacle is present in *G. aburamae* Yamaguti 1934, *G. anurus* (Layman 1930) and in the two species described herein.

The ventral sucker has the same appearance in ventral view as does that of *G. robusta* and therefore undoubtedly possesses the peculiarity of structure to be described in connection with that species.

A distinct metraterm appears to be present. This structure also occurs in *G. robusta* and *G. manteri.*

Aside from these additions no other change need be made in the description of the species as given by Manter except to note that the flattened body form which he attributed to this species is probably due to killing under pressure.

Genolinea robusta n. sp.

Figures 11–15

About 100 specimens of this worm were found in the stomach of a "marbled sculpin," *Scorpaenichthys marmoratus* (Ayres), taken at False Bay on San Juan Island (collection of Dr. J. E. Guberlet No. 979: 536) and a few additional specimens were secured from two "ling cod," *Ophiodon elongatus* (Girard), taken in Seattle.

The species, as the specific name suggests, is of thick-set muscular body form, the pre-soma especially being highly muscular. In unflattened specimens the body is approximately cylindrical, tapering bluntly toward either end. In the living specimen, the highly contractile presoma may extend until it reaches a length nearly equal to that of the post-soma and is then narrow and tapering. The cuticle is thick and thrown into folds in contracted specimens. In killing, more or less contraction invariably occurs, the pre-soma bends ventrally and a constriction of the body usually occurs just anterior to the ventral sucker and frequently also just posterior to it. The following description is based on the material from *Scorpaenichthys marmoratus.*

Length 1.8–2.4 mm, width 0.35–0.60 mm. Oral sucker, overhung by prominent lip, 0.17–0.20 × 0.15–0.19 mm; pharynx about 0.10 mm in diameter; esophagus short and intestinal ceca of large calibre and reach to posterior end; ventral sucker 0.30–0.36 mm in diameter, its anterior border 0.4–0.6 mm from anterior end. Testes transversely ovate, one behind other in posterior end of middle third of body, anterior testis 0.20–0.27 × 0.11–0.17 mm, posterior 0.21–0.30 × 0.13–0.17 mm; seminal vesicle much coiled extending posteriorly to or slightly beyond anterior edge of ventral sucker; pars prostatica short and dilated into globular form distally; genital sinus enlarged at base and surrounded by very muscular, pear-shaped sinus sac (figs. 12, 14); genital pore median, slightly posterior to pharyngeal level. Ovary and vitellaria transversely ovate in anterior part of posterior third of body; ovary 0.22–0.32 × 0.15–0.18 mm, anterior vitellarium 0.20–0.27 × 0.11–0.16 mm, posterior vitellarium 0.17–0.26 × 0.10–0.16 mm; seminal receptacle large, anterior and dorsal to ovary; (see fig. 15 for structure of ovarian complex); muscular metraterm begins about at anterior border of ventral sucker; eggs 28–36 × 15–18 microns. Excretory system of usual hemiurid type with branches uniting dorsal to pharynx; parenchyma densely fibrous in appearance. The specimens from *Ophiodon elongatus* agree in every respect except for possibly a more globular shape of the vitellaria.

Host.—*Scorpaenichthys marmoratus* (Ayres), marbled sculpin.
Location.—Stomach.
Locality.—Friday Harbor, Washington.
Type.—U.S.N.M. No. 9075.

The structure of the ventral sucker in this species deserves special mention. It is of globular form and has a purse-shaped appearance in ventral view, its aperture being partly closed by thickened, flap-like extensions of the cuticle of the body wall. A striking peculiarity is the presence of a thick ring of muscle running around the aperture of the sucker and embedded in the usual prismatic musculature (fig. 14). Seen in sagittal section, either actual or optical, the sucker appears to be knobbed. Presumably this muscular ring acts as a sphincter.

Genolinea robusta is similar to *G. laticauda* Manter. It differs only in the shape of the gonads which are decidedly wider than long in *G. robusta,* globular in *G. laticauda* and in possessing a generally more robust, muscular body. The seminal receptacle in the one specimen of *G. laticauda* examined is to the left of the ovary whereas in *G. robusta* it is medial and anterior to the ovary. This position in *G. laticauda* may, however, be due to killing under pressure.

Genolinea manteri n. sp.

Figures 16 and 17

The specimens on which this description is based were from the stomachs of several "smooth sculpins," *Leptocottus armatus* (Girard), taken in Seattle. As the anatomy differs only in details from that of *Genolinea robusta,* a briefer description, confined principally to points of difference between the two species, will suffice.

Genolinea manteri n. sp., is about the same size but decidedly less muscular than *G. robusta.* In length it varies from 1.75 to 2.10 mm and in width from 0.32

to 0.45 mm. Dimensions of oral sucker are 0.12 to 0.14 mm by 0.11 to 0.15 mm; of ventral sucker 0.26 to 0.33 mm by 0.26 to 0.32 mm. Pharynx and digestive tract are as in *G. robusta.* The ventral sucker differs from that of *G. robusta* in being less deeply set and in lacking the sphincter muscle.

All of the genital glands are relatively smaller than those of *G. robusta* and are nearly globular rather than transversely ovate. Measurements expressed as length times width are:

Anterior testis	0.09–0.15 × 0.12–0.15 mm
Posterior testis	0.12–0.20 × 0.11–0.20 mm
Ovary	0.10–0.16 × 0.12–0.16 mm
Anterior vitellarium	0.12–0.15 × 0.10–0.17 mm
Posterior vitellarium	0.11–0.16 × 0.12–0.15 mm
Eggs	35–38 × 19–20 microns

Details of the female reproductive system including shell gland complex, course of uterus and position of seminal receptacle as in *G. robusta.* The metraterm begins at about the anterior border of the ventral sucker. Likewise, the male system is essentially as in *G. robusta* with the exception that the seminal vesicle extends to the middle of the ventral sucker (fig. 17). The sinus sac differs only in being slightly less muscular and the genital sinus apparently lacks the muscular dilatation at its base. As in *G. robusta* the genital pore is median, just behind the pharynx.

Host.—*Leptocottus armatus* (Girard), smooth sculpin.

Location.—Stomach.

Locality.—Seattle, Washington.

Type.—U.S.N.M. No. 9076.

Subfamily Hemiurinae

Hemiurus levinseni Odhner 1905

Figures 18 and 19

The material on which this study is based is from the collection of Dr. J. E. Guberlet (Nos. 991: 548 and 1038: 589), the host in both cases being the spring salmon, *Oncorhynchus tschawytscha* (Walbaum). Kohlruss (1933) reported *H. levinseni* also from *Sebastodes ruberrimis* Cramer, *Ophiodon elongatus* (Girard) and *Sebastodes caurinus* (Richardson). The parasite inhabits the stomach and occurs with *Brachyphallus crenatus* (Rudolphi) in the salmon.

Hemiurus levinseni is rather easily distinguished from other species of the genus on the basis of sucker ratio. In this species the oral sucker is always slightly larger than the ventral sucker whereas in other members of the genus the ventral sucker is the larger, usually by at least one half. Yamaguti (1934) described the species, *H. odhneri,* which has a larger oral sucker and is in other respects similar to *H. levinseni.* Yamaguti distinguished his species from *H. levinseni* on the basis of the following differences: (a) larger size, of 2.3–5 mm, Odhner (1905) giving 1.0–1.6 mm as the limits for *H. levinseni;* (b) uterus in *H. odhneri* not passing between the testes; and (c) the seminal vesicle directed dorsoventrally; transversely in *H. levinseni.*

These are rather slight differences but if constant are probably sufficient for specific distinction. Material from the salmon agrees in all respects with the description of *H. levinseni* as given by Odhner (1905)

and Manter (1926) and would tend to confirm the constancy of the specific characters of the species as given by Odhner. Measurements on a typical specimen from the salmon for comparison with the limits established by Odhner are as follows:

	Odhner	*From salmon*
Length	1–1.6 mm	1.25 mm
Width	1/3 length	0.39 mm
Oral Sucker	0.14–0.20 mm	0.16 mm
Ventral Sucker	0.11–0.17 mm	0.14 mm
Eggs	26–28 × 12–13 microns	26 × 12 microns

Mention should be made of the presence of a short but well differentiated metraterm in this species (fig. 19). Looss (1907: 96) stated that forms with a "true cirrus sac" lack a metraterm but, while short, a definite metraterm is present in this species.

Parahemiurus platichthyi n. sp.

Figure 20

A single specimen of a species of *Parahemiurus* from the stomach of the "starry flounder," *Platichthys stellatus rugosus* Girard (collection of Dr. Guberlet No. 996: 553), forms the basis of this description. While only a single specimen is available it has been possible to ascertain its anatomy in a fairly satisfactory fashion, an account of which follows.

Length 1.3 mm, width 0.32 mm; cuticular rings extend ventrally to near base of ecsoma, dorsally to middle of posterior testis; preacetabular region 0.1 mm and completely invaginated ecsoma 0.2 mm in length; oral sucker 0.06 × 0.07 mm and ventral sucker 0.14 × 0.14 mm, ventral sucker relatively massive and projecting forward from ventral surface. Testes and ovary both transversely ovate with following measurements; anterior testis 0.13 × 0.11 mm, posterior testis 0.14 × 0.10 mm; ovary 0.125 × 0.085 mm; testes close together, obliquely one behind other in anterior part of middle third of body; both yolk glands appear to be nonlobed; genital pore at postero-ventral edge of oral sucker and from it sinus sac extends to posterior edge of ventral sucker; slightly sinuous pars prostatica reaches to anterior testis and the oval and apparently undivided seminal vesicle lies dorsal to posterior end of pars prostatica, slightly overlapping anterior testis; eggs measure 21–23 × 10–11 microns.

Host.—*Platichthys stellatus rugosus* Girard, starry flounder.
Location.—Stomach.
Locality.—Friday Harbor, Washington.
Type.—U.S.N.M. No. 9078.

Vaz and Pereira (1930) established the genus *Parahemiurus* on the basis of an undivided seminal vesicle. Linton (1911) described *Hemiurus merus* in which the seminal vesicle is also stated to be undivided and Manter (1934) recorded *Hemiurus* sp. which likewise shows this character. *Parahemiurus parahemiurus* Vaz and Pereira, *Hemiurus merus* Linton and *Hemiurus* sp. Manter are all similar to the present specimen from the flounder. Manter's specimen differs in possessing a slightly larger oral sucker, shorter sinus sac and prostate, more posterior ventral sucker, lobed yolk glands, and slightly smaller eggs (18 × 9–10 microns).

Hemiurus merus Linton differs in the following respects; it is stated that dorsal cuticular rings do not extend to the ventral sucker; the ventral sucker appears from Linton's figure to be slightly more posterior, the ecsoma is longer and the right yolk gland lobed (Linton's figure shows the right gland as lobed although his text states it is the left). The eggs of *H. merus* are also somewhat larger, 27×10 microns.

Vaz and Pereira said of *P. parahemiurus* "cuticle ruguense, a une dentelure transversale sans le tiers anterieur du corps"; no figure was presented. Presumably, the cuticular ringing is limited to the anterior third of the body which is true of its dorsal extent in this specimen from the flounder but not of its ventral or lateral distribution. Other respects in which Vaz and Pereira's species differ are slight. Its size is somewhat larger but certainly within the range of specific variation and the relative size and position of suckers, size of pharynx, extent of intestinal ceca and uterus and size of ecsoma are nearly the same in both, the suckers being significantly smaller in *P. platichthyi*. The testes appear to be located more posteriorly and are relatively smaller, the ovary is not significantly different while the eggs are somewhat larger, 24×10–14 microns as compared to 21–23×10–11 microns in *P. platichthyi*.

Yamaguti (1934) described two species of *Parahemiurus, P. sardiniae* and *P. seriolae*. Of these *P. sardiniae* is somewhat similar to *P. platichthyi* but differs markedly in the more posterior position of the ovarian complex and the extent of intestinal ceca and uterus into the ecsoma.

Additional information concerning the anatomy of *Parahemiurus parahemiurus* Vaz and Pereira and *P. platichthyi* might readily prove them identical. The question as to the advisability of recognizing the genus *Parahemiurus* as distinct from *Hemiurus* on the sole basis of the possession of an undivided seminal vesicle, while somewhat subjective, also requires additional information concerning the several species exhibiting that character for its answer. Manter (1934) has already expressed doubt as to the validity of the genus *Parahemiurus*. If the seminal vesicle proves, on closer study, to be completely undivided in the several species, recognition of the genus would appear to be justifiable.

For the present it seems best to recognize the genus with *Parahemiurus platichthyi* distinguished from *P. parahemiurus* by the difference in distribution of the cuticular rings, by its relatively larger testes and smaller suckers.

Intermediate between Hemiurinae and Sterrhurinae

Brachyphallus crenatus (Rudolphi 1802) Odhner 1905

Figures 21–25

This species was obtained from the stomachs of "spring salmon," *Oncorhynchus tschawytscha* (Walbaum), where it occurs with *Hemi-*

urus levinseni.. Yamaguti (1934) reported the species from *O. milktschitsch* (Walbaum) and Odhner (1905) likewise found it in Atlantic salmon, *Salmo salar* Linnaeus.

This species has been the subject of a thorough morphological study by Lander (1904) and it will, therefore, be unnecessary to consider its anatomy in detail. There are a few points of disagreement between Lander's description and the present study as well as the question of the validity of the species *Brachyphallus affinis* which Looss (1907) established for the American species on the basis of Lander's work.

The genus *Brachyphallus* shows a combination of characters which would ally it with the HEMIURINAE on the one hand and the STERRHURINAE on the other. These characters are the ringed cuticle of the HEMIURINAE and the pre-somatic pit and decidedly vesicular parenchyma of the STERRHURINAE. The structure of the terminal genital ducts will be considered separately. Looss (1907) recognized this intermediate position of *Brachyphallus* and therefore placed it between the HEMIURINAE and STERRHURINAE. As Looss has stated, Odhner (1905) included *Brachyphallus* with the HEMIURINAE as likewise did Fuhrmann (1928). In his key to HEMIURIDAE, Looss (1907) placed *Brachyphallus* with the STERRHURINAE because of the lack of a complete sinus sac. Odhner (1905: 354), however, described a complete sinus sac in *Brachyphallus crenatus* and Lander (1904: Plate 3, fig. 28) showed a similar structure as present in his material. This study has revealed a small but complete sinus sac surrounding the genital sinus (fig. 24). In this respect the terminal genital duct has the structure of the HEMIURINAE, although by the presence of a distinct ejaculatory duct and short prostate *Brachyphallus* resembles the STERRHURINAE.

The author finds it impossible to assign the genus *Brachyphallus* to either the HEMIURINAE or STERRHURINAE and is in agreement with Looss that it is best to consider it as an isolated genus intermediate between the two subfamilies.

This study is in nearly perfect agreement with Lander's description but a few points of difference are worthy of mention. Lander figured (Plate 3, fig. 28) the uterus as continuing completely undifferentiated into the genital sinus. Odhner (1905: 354 and Taf. IV, fig. 4) was not clear on this point but Looss (1907: Taf. 14, fig. 65) showed a short, differentiated terminal portion of the uterus. Incidentally, Looss' generic diagnosis of *Brachyphallus* described a long metraterm as present which is not true of *B. crenatus* although in the present material the uterine wall appears to become slightly more muscular at about the level of the anterior testis. Besides this there is a short but well differentiated metraterm (fig. 24M).

The cells which Lander (1904: 9) described and figured (Plate 2, fig. 25) as enclosing the posterior ends of the intestinal ceca do not occur in sections of the present material.

The shape of the vitellaria should be mentioned since it is upon this point that Looss based the species *Brachyphallus affinis*. Lander (p. 22) described them as irregular oval bodies with their longest diameter parallel to the longitudinal axis of the body, commonly slightly lobulated but sometimes with a regular oval outline. Manter (1926) expressed doubt that the American form is distinct from *B. crenatus* and stated that his material from *Osmerus mordax* the same host from which Lander obtained his material, agreed perfectly with the descriptions of *B. crenatus,* the vitellaria being four and three lobed and not appreciably longer than wide.

The present material agrees in every respect, including the shape of the vitellaria, with the description of *B. crenatus*. It should be pointed out, however, that the vitellaria, when viewed from frontal or dorsal aspect, usually do not show the lobulation completely, and almost invariably appear longer than wide (fig. 25). Distinct lobulation is readily apparent when they are viewed obliquely.

A typical specimen from salmon gives the following measurements: length, 3 mm; width, 0.4 mm; ecsoma, 0.65 mm; oral sucker, 0.32×0.31 mm; ventral sucker, 0.31×0.27 mm; anterior testis, 0.17×0.11 mm; posterior testis, 0.17×0.10 mm; ovary, 0.19×0.21 mm; left vitellarium, 0.21×0.19 mm; right vitellarium, 0.23×0.12 mm; eggs, 26×12 microns.

Subfamily Sterrhurinae

Lecithochirium exodicum MacFarlane 1936

Figures 26–29

This species has been found invariably present in the stomachs of "ling cod," *Ophiodon elongatus* (Girard), taken at Friday Harbor, but not at all at Seattle. These worms have likewise been found in "rock cod," *Sebastodes maliger* (Jordan and Gilbert), at Friday Harbor but are less common in that host. MacFarlane (1936) recorded *Lecithochirium exodicum* from *Ophiodon elongatus* taken in British Columbia waters. His description is somewhat incomplete so that it seems advisable to report upon it more fully.

The genus *Lecithochirium* was established by Lühe (1901) with *Lecithochirium rufoviride* (Rudolphi) as the type species. Looss (1907) diagnosed the genus and also treated of its morphology in greater detail than did Lühe. The genus is separated from the very similar genus *Sterrhurus* by the presence of a pre-somatic pit with underlying cell pad. Looss also gave as a diagnostic feature the presence of two lateral elevations extending into the lumen of the oral sucker. These are not pres-

ent in *Lecithochirium synodi* Manter (1931), *Lecithochirium* sp. Manter (1934) nor in the material at hand.

Observations on the present material show the following dimensions: size, 1.8–4.0 × 0.5–1.0 mm; oral sucker, 0.14–0.26 mm; ventral sucker, 0.34–0.55 mm; testes, 0.16–0.26 mm; ovary, 0.20–0.26 × 0.14–0.18 mm; eggs, 21–25 × 12–14 microns.

Points in addition to MacFarlane's description: smooth cuticle, vesicular parenchyma; oral sucker overhung by prominent lip.

Several structures observed here are in contrast to the work of the above author. He states that the ceca extend into the ecsoma but in the material at hand, the ceca only rarely pass into it. Some question may be raised about the seminal vesicle as MacFarlane states "seminal vesicle convoluted, not bipartite." Observations here show this organ to be tripartite. There is a long metraterm reaching to the middle of the ventral sucker which passes between fibers making up an incomplete sinus sac to enter the genital sinus (fig. 28). A typically hemiurid ovarian complex (fig. 27) is located either on right or left side. The yolk glands are relatively compact but usually lobulate, right being four and left three lobed.

Of particular interest in this genus is the pre-somatic pit already noted (fig. 28). A somewhat similar structure exists in *Brachyphallus* but in this genus it is a transverse, slit-like depression and its underlying structure also differs (figs. 22, 24). In *Lecithochirium,* it is a small circular pit into which frequently projects a cuticular papilla. Muscle fibres radiate from it and beneath these lies a pad of cells. A similar group of cells is also located just beneath the muscle layer between the genital pore and oral sucker.

The literature has revealed no suggestion of a possible function for this structure. The radiating muscle fibres, capable of regulating the aperture of the pit, might suggest that it had the properties of a sucker. However, it appears to be much too small to function effectively as a sucker. The suggestion is here offered that it serves as a chemical sense organ.

Looss suggested (1907: 74) that the presence of an ecsoma in the Hemiuridae is associated with the fact that they are commonly parasites in the stomachs of carnivorous fish. The ecsoma, he thought, is retracted when gastric acidity and enzyme concentration is high and extended at other times to perform the functions of absorption and respiration. It appears at least possible that the pre-somatic pit might serve in detecting chemical changes in the environment and that in this way the retraction or extension of the ecsoma might be controlled.

Lecithochirium exodicum differs from *L. rufoviride* (Rudolphi) and *L. gravidum* Looss in the absence of the elevations of the oral sucker and in its more compact vitellaria. It differs from *L. synodi* Manter in

its much larger eggs and more compact vitellaria and from *Lecithochirium* sp. Manter in more compact vitellaria and in the presence of the cell pad underlying the pre-somatic pit. *L. caudiporum* (Rudolphi) has similar compact vitellaria but the eggs in this species are larger and have a peculiar bean-shaped appearance (Looss, 1907). The specimens from the rock cod agree in all details with those from the ling cod except that the testes seem to average somewhat larger in size, but the difference is too slight for specific distinction.

Park (1936) described a hemiurid, from the stomach of *Citharichthys sordidus* (Girard) in California, which he has designated as *Sterrhurus magnatestis*. This form is very similar to, if not identical with, the species under consideration here. The genus *Lecithochirium* is separated from the closely related genus *Sterrhurus* by the presence of a pre-somatic pit. Park shows such a pit to be present in his *Sterrhurus* which appears to be identical with that of *L. exodicum* MacFarlane and in the present form. However, *S. magnatestis* Park appears to have slightly larger testes and shows a short Laurer's canal.

Subfamily Lecithasterinae

Lecithaster salmonis Yamaguti 1934

Figure 30

Five specimens of this species were obtained from the intestine of the spring salmon, *Oncorhynchus tschawytscha* (Walbaum), (collection of Dr. J. E. Guberlet nos. 991 : 548 and 1038 : 389). The material was in a poor state of preservation and it was not possible to determine with entire accuracy and satisfaction the anatomy of the worm in detail. Particularly is this true of the vitellaria, the lobes of which are shown as considerably thicker than indicated by Yamaguti.

Yamaguti (1934) described *Lecithaster salmonis* from the large intestine of *Salmo keta* (= *Oncorhynchus keta* (Walbaum)), the dog salmon. The following table will serve for comparison of the Puget Sound specimens with Yamaguti's material.

	Puget Sound Material	*Yamaguti*
Length	0.60 to 1.0 mm	1.1 to 1.26 mm
Width	0.27 to 0.39 mm	0.42 to 0.52 mm
O. S.	0.08–0.11 × 0.08–0.115 mm	0.063–0.084 × 0.095–0.13 mm
V. S.	0.14–0.18 × 0.14–0.17 mm	Average 0.18 mm
Testes	0.089 × 0.063 mm	0.12 × 0.11 mm
Eggs	21–24 × 14–16 microns	24 × 16 microns

Aside from the generally smaller size and smaller testes the examples from the spring salmon agree well with the description of the species as given by Yamaguti. When the similarity of hosts is also considered

there appears to be little doubt of the identity of the material from the two localities.

Subfamily Syncoelinae

Syncoelium filiferum (Sars 1885)

This parasite from the gills of salmon has been described elsewhere (Lloyd and Guberlet, 1936). No additional data are available.

Subfamily Accacoeliinae

Odhnerium calyptrocotyle (Monticelli 1893)

Figures 31–35

About two dozen specimens of this species were secured from the intestine of a "sunfish," *Mola mola* (Linnaeus). The host taken off the west coast of Vancouver Island, August 14, 1934, had been dead for several days and no trematodes were alive.

The original description of *Distomum calyptrocotyle* Monticelli (1893) was based on relatively immature specimens from *Beroe ovata* Chamisso and Eysenhardt. Linton (1898) described a species *Distomum foliatum* from *Mola mola* which Odhner (1928) identified with Monticelli's *D. calyptrocotyle*. Odhner stated that this species formed a fourth genus of Accacoeliidae but did not himself establish a genus for it. Looss (1902) also noted the similarity between Monticelli's and Linton's species and provisionally placed them both in the genus *Orphocotyle*, but as separate species. Yamaguti (1934), after a study of material also from *Mola mola,* agreed with Odhner and erected the genus *Odhnerium* to contain this species with *Odhnerium calyptrocotyle* (Monticelli) as type.

Monticelli's description of *D. calyptrocotyle* is very complete while Linton's of *D. foliatum* is quite meagre and contains a number of obvious errors. There appears, however, to be little doubt of the identity of the two, especially since Odhner had some of Linton's material for comparison with the European form. As Yamaguti has also described this species in considerable detail it will be unnecessary to do so here except for the following observations.

Laurer's canal which Monticelli was unable to find in his material is present as described by Yamaguti. Linton described "six or eight nodular eminences on the dorsum of head and neck" where actually there are eleven as noted by Yamaguti. Of these the most anterior one, which overhangs the oral sucker in a lip-like fashion (fig. 32) differs from the rest in lacking a distinct limiting membrane, the muscle fibres of which it is composed are attached to the body wall and oral sucker. Linton described a "cirrus bulb" as present. No cirrus pouch is present but the muscular genital sinus is capable of being evaginated as a functional

cirrus. Two prominent bands of retractor muscle fibers extend from its anterior and posterior walls to the body wall (fig. 34). The eggs in the present material measure 34 × 20 microns.

HEMIURID LARVA

Two specimens of a larval hemiurid were obtained from the ctenophore, *Bolinopsis microptera* (A. Agassiz). The worms measure about 0.5 mm. in length, have a smooth cuticle and small ecsoma. The presence or absence of a pre-somatic pit could not be determined. They are probably a species of *Sterrhurus* or *Lecithochirium,* possibly *Lecithochirium exodicum.*

SUMMARY

1. The following digenetic trematodes are reported and described from Puget Sound fish.

Family MONORCHIIDAE Odhner
Subfamily PROCTOTREMINAE
Telolecithus pugetensis Lloyd and Guberlet 1932
Family ALLOCREADIIDAE Stossich
Subfamily ALLOCREADIINAE
Cymbephallus vulgaris Manter 1934
Subfamily OPECOELIINAE•
Opecoelina theragrae n. sp.
Family AZYGIIDAE Odhner
Otodistomum veliporum (Creplin 1842)
Family HEMIURIDAE Lühe
Subfamily DEROGENETINAE
Derogenes varicus (O. F. Müller 1784)
Genolinea robusta n. sp., *G. manteri* n. sp.
Subfamily HEMIURINAE
Hemiurus levinseni Odhner 1905
Parahemiurus platichthyi n. sp.
Subfamily STERRHURINAE
Lecithochirium exodicum MacFarlane 1936
Intermediate between HEMIURINAE and STERRHURINAE
Brachyphallus crenatus (Rudolphi 1802)
Subfamily LECITHASTERINAE
Lecithaster salmonis Yamaguti 1934
Subfamily SYNCOELIINAE
Syncoelium filiferum (Sars 1885)
Subfamily ACCACOELIINAE
Odhnerium calyptrocotyle (Monticelli 1893)

2. A larval hemiurid is reported from the ctenophore *Bolinopsis microptera* (A. Agassiz).

3. A suggestion is offered as to a possible function of the pre-somatic pit of the STERRHURINAE.

4. A striking variation of the incidence of infection of *Cymatogaster aggregatus* with *Telolecithus pugetensis* is reported and a suggestion offered as to a possible contributory cause.

5. A similarity between the parasitic fauna of *Oncorhynchus tschawytscha* and *Salmo salar* is reported.

6. The possibility of a decided difference between the parasitic fauna of littoral fish from the San Juan region and the Puget Sound region proper is indicated.

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EXPLANATION OF PLATES

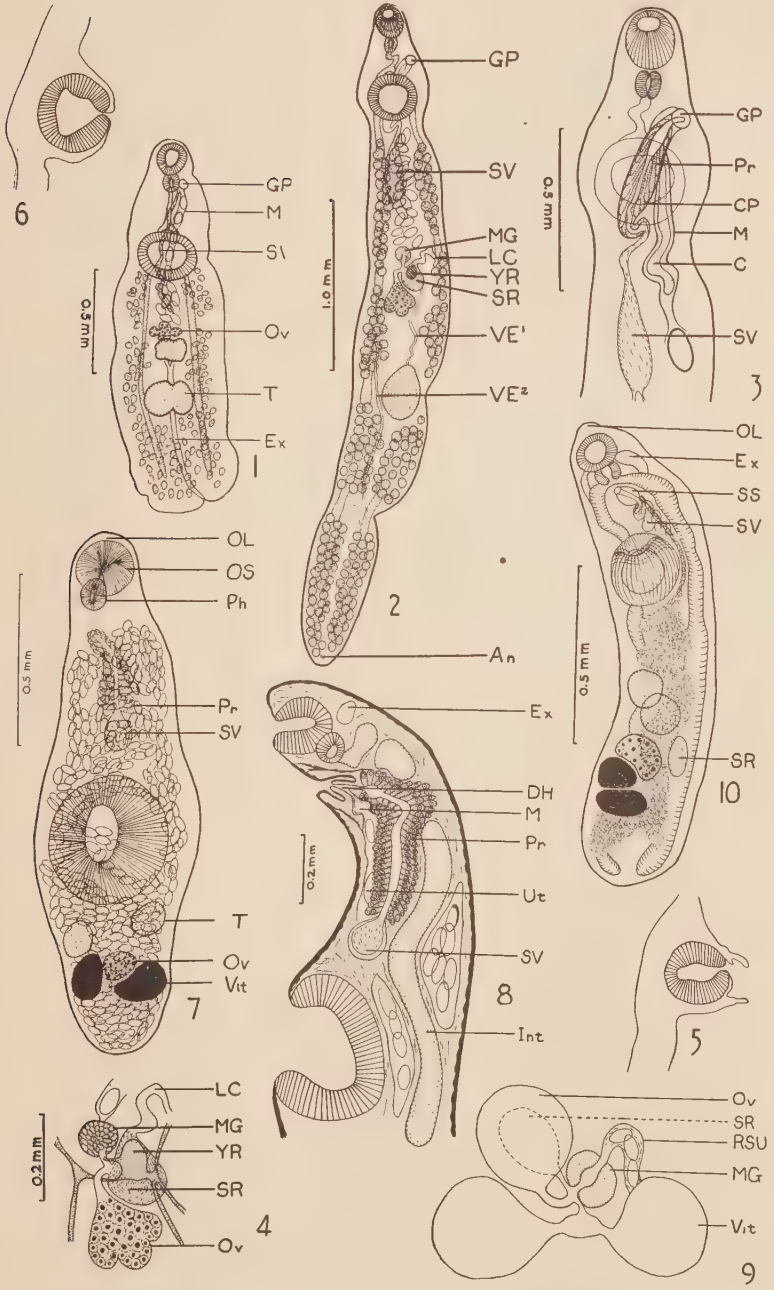
All drawings, except those labelled diagrammatic, were made with the aid of a camera lucida. The projected scale has its value indicated in all cases.

Abbreviations Used in Plates

An	Anus	Ph	Pharynx
C	Cirrus	PP	Pars prostatica
CMB	Circular muscle bundle	Pr	Prostate
CP	Cirrus pouch	Pre	Pre-pharynx
DH	Ductus hermaphroditus	PSP	Pre-somatic pit
Ec	Ecsoma	R	Reduplication of ventral sucker
ED	Ejaculatory duct	RSU	Uterine seminal receptacle
Ex	Excretory	SR	Seminal receptacle
GP	Genital pore	SS	Sinus sac
Int	Intestine	SV	Seminal vesicle
LC	Laurer's canal	T	Testis
M	Metraterm	VE	Vasa efferentia
MG	Mehlis' gland	Vit	Vitellaria
Od	Oviduct	VP	Pre-somatic pit
OL	Oral lip	VS	Ventral sucker
OS	Oral sucker	YD	Yolk duct
Ov	Ovary	YR	Yolk reservoir
P	Muscular papilla		
Par	Parenchyma		

Explanation of Plate I

Fig. 1. *Cymbephallus vulgaris* Manter 1934, ventral view of whole mount.
Figs. 2–6. *Opecoelina theragrae* n. sp.
Fig. 2. Ventral view of whole mount.
Fig. 3. Reconstruction of terminal genital ducts.
Fig. 4. Semi-diagrammatic reconstruction of ovarian complex.
Fig. 5. Ventral sucker, sagittal section lateral to median plane.
Fig. 6. Ventral sucker, sagittal sections through median plane.
Figs. 7–9. *Derogenes varicus* (O. F. Muller 1784).
Fig. 7. Dorsal view of whole mount.
Fig. 8. Reconstruction of anterior end.
Fig. 9. Ovarian complex, diagrammatic.
Fig. 10. *Genolinea laticauda* Manter 1925, drawn from Manter's paratype.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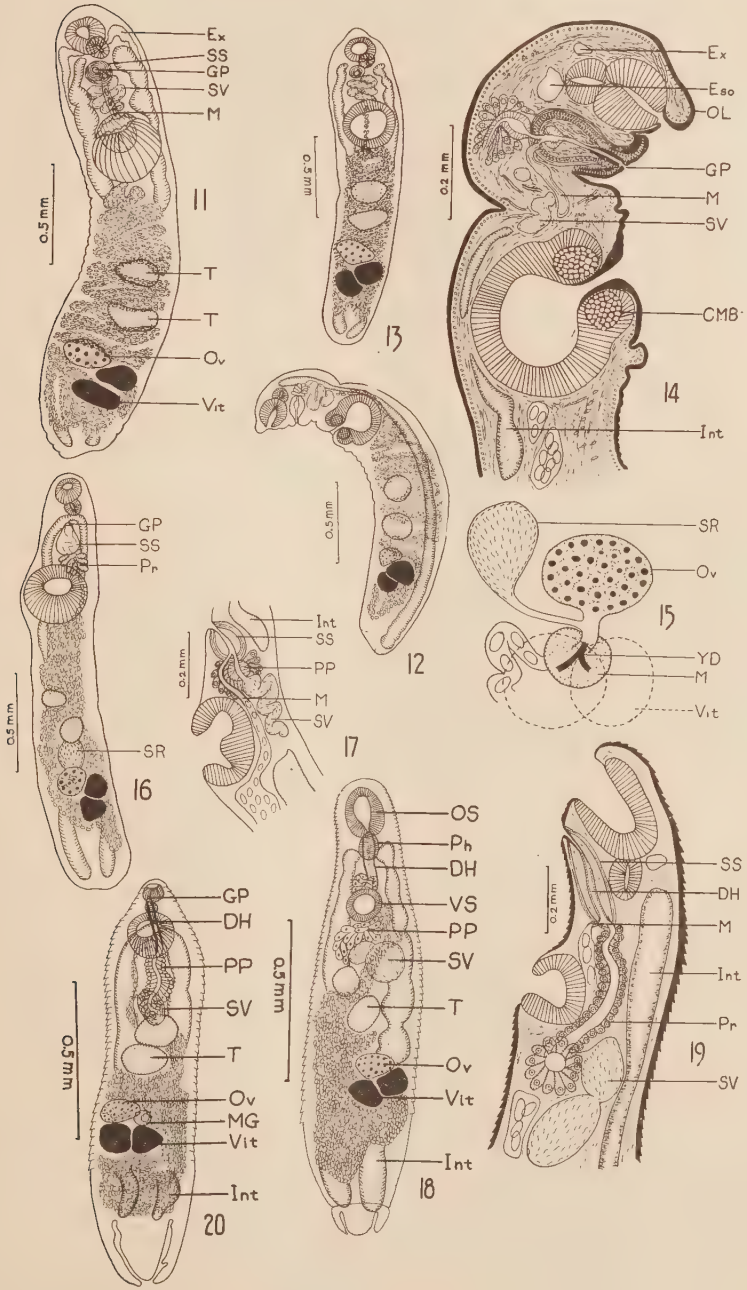
Explanation of Plate II

Figs. 11–15. *Genolinea robusta* n. sp.
Fig. 11. Typical specimen from *Scorpaenichthys marmoratus,* ventral view.
Fig. 12. Lateral view of specimen from *S. marmoratus.*
Fig. 13. Specimen from *Ophiodon elongatus,* ventral view.
Fig. 14. Reconstruction of anterior end.
Fig. 15. Ovarian complex, diagrammatic.
Figs. 16, 17. *Genolinea manteri* n. sp.
Fig. 16. Ventral view of whole mount.
Fig. 17. Reconstruction of anterior end.
Fig. 18. *Hemiurus levinseni* Odhner 1905, whole mount from ventral view.
Fig. 19. *H. levinseni* Odhner, reconstruction of anterior end.
Fig. 20. *Parahemiurus platichthyi* n. sp., ventral view of whole mount.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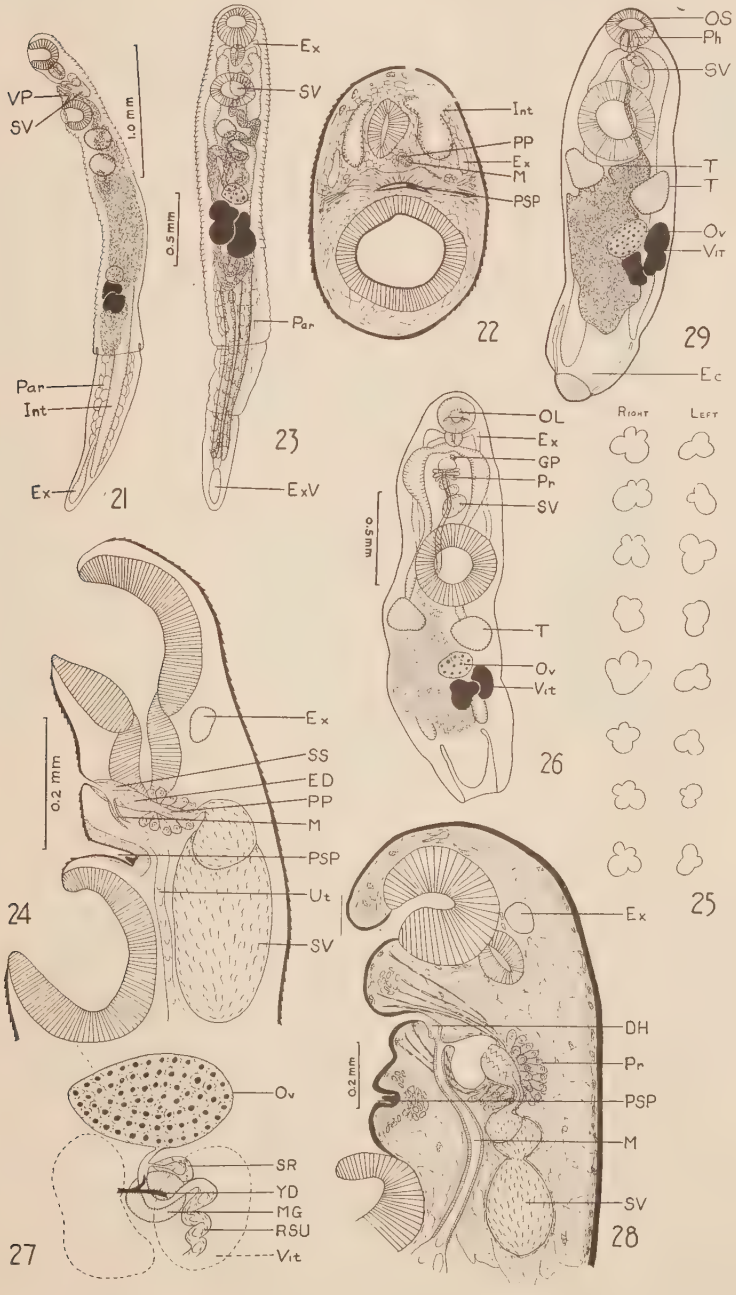
Explanation of Plate III

Figs. 21–25. *Brachyphallus crenatus* (Rudolphi 1802).
Fig. 21. Lateral view from whole mount.
Fig. 22. Semi-frontal section of anterior region.
Fig. 23. Ventral view from whole mount.
Fig. 24. Reconstruction of anterior end.
Fig. 25. Outline drawings of shape of vitellaria.
Figs. 26–29. *Lecithochirium exodicum* MacFarlane 1936.
Fig. 26. Ventral view of whole mount from *Ophiodon elongatus.*
Fig. 27. Ovarian complex, diagrammatic.
Fig. 28. Reconstruction of anterior end.
Fig. 29. Somewhat abnormal specimen from *Sebastodes maliger,* ventral view.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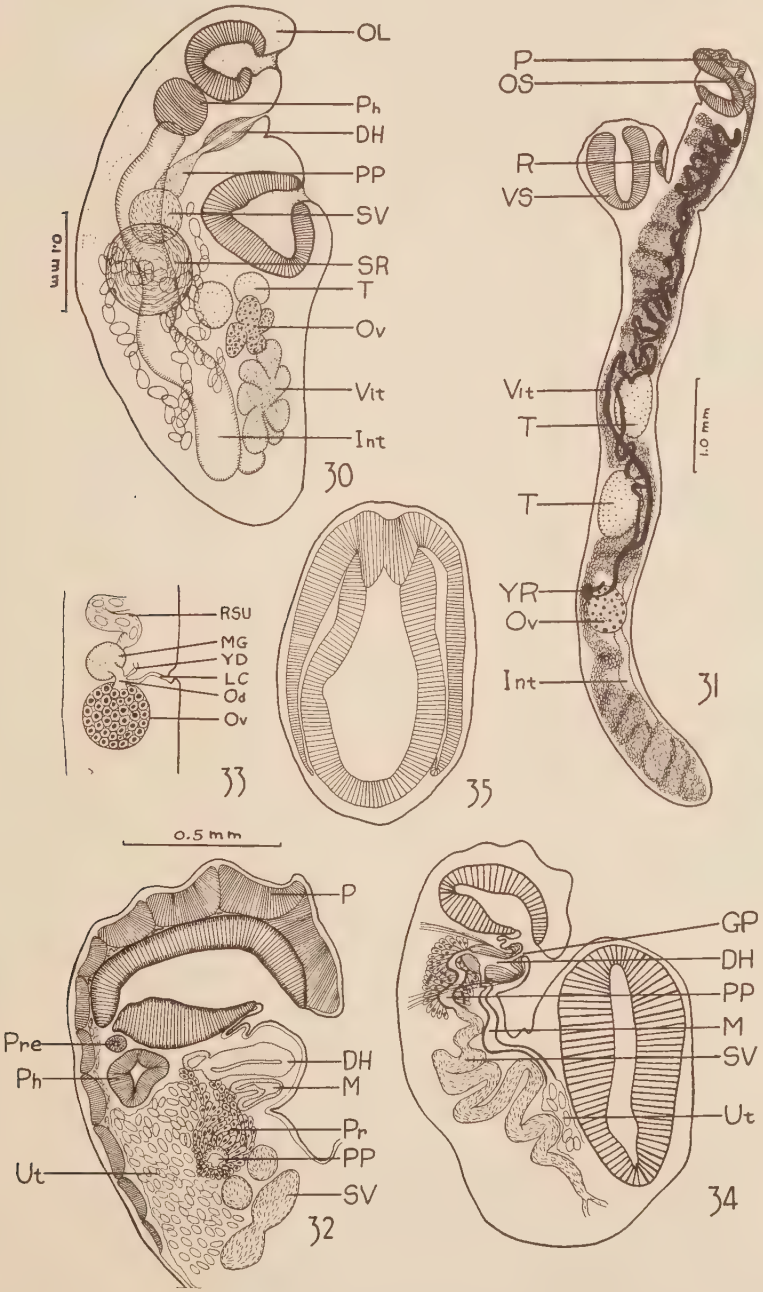
Explanation of Plate IV

Fig. 30. *Lecithaster salmonis* Yamaguti 1934, lateral view.
Figs. 31–35. *Odhnerium calyptrocotyle* (Monticelli 1893).
Fig. 31. Lateral view of whole mount.
Fig. 32. Sagittal section through anterior end.
Fig. 33. Ovarian complex, diagrammatic.
Fig. 34. Reconstruction of anterior end.
Fig. 35. Section through ventral sucker.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TANAISIA PELIDNAE N. SP. AND *ORCHIPEDUM TRACHEICOLA* (TREMATODA)[1]

E. L. Cheatum[2]

A new trematode of the genus *Tanaisia,* family Eucotylidae Skrjabin is here described. *Orchipedum tracheicola* Braun, family Orchipedidae Skrjabin is discussed as the first member of the family to be reported from North America and the second report on the species since the original description by Braun (1901).

genus *Tanaisia* skrjabin 1924

The family Eucotylidae was established by Skrjabin (1924), to include *Eucotyle* Cohn (1904), *Tamerlania* Skrjabin (1924), and *Tanaisia* Skrjabin (1924). To these were added the genera *Ohridia* and *Lepidopteria* by Nezlobinski (1926). The genus *Tanaisia* contains three species, *T. fedtschenkoi* Skrjabin, *T. elliptica* Nezlobinski, and *T. rossitensis* Korkhaus. Skrjabin lists for *T. fedtschenkoi* eleven hosts, *Himantopus candidus, Chettusia leucura, Totanus glottis, T. ochropus, Tringa minuta, Hydrochelidon nigra, Sterna fluviatilis, S. anglica, Chroicocephalus ridibundus, Larus canus,* and *Rallus aquaticus.* They were collected in Turkestan and in the region of the Don River in the southern part of the U.S.S.R. *T. elliptica* is described from *Hydrochelidon nigra* collected in the Ohrid basin, Macedonia. *T. rossitensis* was taken from *Corvus cornix,* and is the only member of the genus described from other than shore-birds. It was collected near Rossiten, Germany.

Issaitschikoff (1926) added several salient features to the generic diagnosis, the most important being the union of the intestinal crura in the posterior end of the body. The species herein described falls within the genus with the exception of one character, the testes are not oblique, but tandem. Since this probably does not constitute a generic difference, the generic diagnosis is accordingly amended to read: Eucotylidae. Medium sized monostomate trematodes with attenuated bodies; head region not separated from remainder of body by muscular collar; esophagus present; testes with margins entire or strongly lobed, between crura and in middle third of body, oblique *or tandem;* ovary anterior to testes with margin entire or strongly lobed; vitellaria marginal, extracecal, in middle third of body; intestinal crura united in hind part of body; parasitic in urinary tract of birds.

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[1] Contribution from the Museum of Zoology and the Department of Zoology, University of Michigan.

[2] I wish to express my appreciation to Prof. G. R. LaRue of the Zoology Department for his criticism in the preparation of this manuscript, to Dr. E. W. Price and Mr. Allen McIntosh of the Bureau of Animal Industry for their assistance in locating literature pertinent to this paper, and to Dr. Pierce Brodkorb of the Museum of Zoology for identification of the birds.

Tanaisia pelidnae n. sp. (Fig. 4)

Two specimens were found in washings from the body cavity of a red-backed sandpiper, *Pelidna alpina-sakhalina* (Vieillot), collected by J. Wood, Sand Point, near Caseville, Michigan, May 22, 1934.

Description of type:

Body flattened, long, elliptical, 3.08 mm long by 0.44 mm wide at level between testes; oral sucker longer than broad, 0.21 × 0.20 mm; pharynx broader than long, 0.075 × 0.094 mm; esophagus with bulbous enlargement, 0.075 mm long; intestinal crura united posteriorly at about 6/7 the body length; testes median, in middle third of body, tandem, strongly lobed, nearly contiguous, extreme length of anterior testis, 0.289 mm, of posterior testis, 0.259 mm; ovary elongate, 0.252 × 0.157 mm, slightly lobate on left, anterior margin, with single lobe extending anteriorly from right posterior margin; vesicula seminalis adjacent to right, anterior border of ovary, 0.090 × 0.071 mm; receptaculum seminis adjacent to left, posterior border of ovary; vitellaria lateral, extra-cecal, occupying middle third of body, right gland 1.37 mm long, left gland 1.45 mm, terminating obliquely anteriorly and posteriorly; vitelline reservoir median, between ovary and first testis; uterus much coiled, descending and ascending limbs filling body posterior to testes, ascending limb crossing to right between first testis and ovary, continuing to pharyngeal region in compact coils filling body between ovary and pharynx, and descending from pharyngeal region toward ovary; genital pore undetermined; eggs operculate, symmetrically oval, 0.033–0.034 × 0.020 mm.

Type specimen: U. S. Nat. Mus. Helm. Coll. No. 8961.

The paucity of specimens prevented sections being made. In the mounted specimens the exact location of the genital pore could not be determined because of the interference of transmitted light by the large number of eggs in the anterior part of the body. Unfortunately the range of variation of the structures used in differentiating this form from described species cannot be determined from two specimens.

It was noted earlier that the parasites were taken from washings of the body cavity, consequently their original location in the host is still a question. Since the related forms are all parasites of the urinary tract, it seems reasonable to assume that these specimens escaped from the ureters or kidneys during evisceration. A close examination of the stained and mounted specimens revealed numerous crystalline bodies in those parts of the digestive system not obscured by eggs. Since there was a possibility that the crystals had been derived from nitrogenous wastes of the host, they were examined by polarized light. Although the identity of the crystals was not revealed, the method aided materially in tracing the major features of the digestive system. The thin walls of the intestine are differentiated poorly by staining. This fact, coupled with the great number of eggs, made it difficult to locate the intestinal bifurcation at the esophagus and the place of union in the posterior part of the body. But these points and the entire digestive system, when viewed with polarized light, were clearly indicated by the contained crystals.

There is a question of synonymy involving *T. rossitensis* with the type species *T. fedtschenkoi*. Korkhaus (1930) described a kidney trematode from *Corvus cornix* under the name *Prohystera rossitensis*. He

discussed its similarity to *T. fedtschenkoi* but distinguished it from the latter by the union of the intestinal crura posteriorly. He apparently was unaware of Issaitschikoff's addition to Skrjabin's description of the genus. Ejsmont (1931) brought *P. rossitensis* into synonymy with *T. fedtschenkoi.* Korkhaus (1935) does not refer to Ejsmont's paper and continues to consider *T. rossitensis* distinct from *T. fedtschenkoi.* With the exception of a detailed description of the egg, he does not elaborate further on his original description. Since Korkhaus' description of *T. rossitensis* appeals to me as insufficient, and the characters which he has described adequately do not differ sufficiently from *T. fedtschenkoi* to warrant a new species, I follow Ejsmont in considering *T. rossitensis* to be a synonym of *T. fedtschenkoi.*

Nezlobinski's description of *T. elliptica* does not conform to the generic diagnosis of *Tanaisia* as amended by Issaitschikoff. He describes the intestinal crura extending to the posterior end of the body where their *blind ends converge somewhat.* It is possible that here, too, the union of the intestinal crura was obscured by eggs, and, unaware of this characteristic of *Tanaisia,* Nezlobinski assumed the converging ends of the crura to end blindly. Since all other characters of *T. elliptica* conform to the generic diagnosis, it is my belief that he inadvertently failed to describe completely the intestinal crura. Until this explanation is disproved, there is not sufficient justification for excluding his species from the genus.

Because of the extreme difficulty in securing some of the literature dealing with this genus and in order to facilitate comparison, it seems advisable to list in some detail the distinguishing characters of the species.

T. fedtschenkoi: Body long, elliptical, 3.9 × 0.8 mm; sucker transversely oval, 0.19 × 0.27 mm; pharynx 0.09 × 0.11 mm; esophagus with bulbous enlargement; intestinal crura united posteriorly at about 7/8 body length; testes oblique, near middle of body, strongly lobed, lobes finger-shaped; ovary median, lobed, anterior to first testis; vesicula seminalis median, adjacent to anterior border of ovary; Mehlis' gland and 2 to 3 coils of uterus between first testis and ovary; vitelline glands extra-cecal, occupying middle third of body, 1.6 mm long; uterine coils fill body posterior to testes and region between ovary and pharynx; eggs elongate, 0.043 × 0.020 mm.

T. elliptica: Body medium length, elliptical, 2.00 × 0.75 mm; sucker round, 0.20 mm diameter; esophagus 0.14 mm long, bulbous enlargement at anterior end, 0.12 mm diameter; intestinal crura slightly twisted, extending to posterior end of body; testes oblique, near middle of body, lobes round; ovary irregularly oval, median, anterior to first testis, posterior lateral margin of right testis touches anterior lateral margin of the left; vesicula seminalis round, 0.14 mm diameter, adjacent to right, upper margin of ovary; receptaculum seminis oval, 0.16 × 0.14 mm, adjacent to lower left margin of ovary, lateral margin partly covers left intestine; vitelline glands not of same length, left gland 0.94 mm long, right 1.11 mm, both beginning at a level with the anterior end of the first testis; uterus making a coarse net-work along the whole body of the parasite; eggs oval, 0.025 × 0.015 mm.

T. pelidnae differs from the two above species in the following characters:

1. Testes are tandem, not oblique as in *fedtschenkoi* and *elliptica.*

2. Ovary is sinistral; it is median in both *fedtschenkoi* and *elliptica.*

3. Vitelline glands terminate at oblique levels both anteriorly and posteriorly, right gland extending forward as far as center of ovary, left gland as far forward as anterior end of ovary; in *fedtschenkoi* and *elliptica* they extend only as far forward as the anterior border of the first testis or posterior border of the ovary.

4. Oral sucker longer than broad; in *fedtschenkoi* it is broader than long, and is round in *elliptica.*

5. Eggs measure 0.033–0.034 × 0.020 mm, whereas the eggs of *fedtschenkoi* are much longer in relation to diameter, their measurement being 0.043 × 0.020 mm; in *elliptica* the eggs are smaller, measuring 0.025 × 0.015 mm.

GENUS *Orichipedum* BRAUN—1901

Orchipedum tracheicola was first diagnosed by Braun (1901) and was later described in some detail in his treatise "Fascioliden der Vogel" (1902), in which he indicated that *Distomum formosum* Sonsino (1890) should be included in the genus *Orchipedum.* He failed, however, to make the combination. Here also he discussed the affinities of *Orchipedum* to *Psilostomum.* Odhner (1913) studied the types of *O. tracheicola* and *Distomum formosum* and placed *formosum* in the genus *Orchipedum,* but definitely excluded this genus from the family Psilostomidae Odhner. Skrjabin (1913), unaware of Odhner's conclusions to the contrary, pointed out the affinities of *Orchipedum* to *Psilostomum,* and divided the family Psilostomidae into two subfamilies, Psilostominae and Orchipedinae, for which he gave diagnoses. At the same time he described *O. turkestanicum* as a new species. Subsequently three more species of *Orchipedum* were described: *O. sufflavum* Nicoll (1914), *O. armeniacum* Skrjabin (1915), and *O. centorchis* Witenberg (1922). Skrjabin (1924) removed the Orchipedinae from the Psilostomidae and elevated it to family rank (Orchipedidae), which included the six members of the single genus *Orchipedum.*

To the best of my knowledge the only work on the life-history of *Orchipedum* is that published by Dollfus, Callot, and Desportes (1935). They found the metacercaria, *Distomum isostoma* Rudolphi, in a species of *Astacus,* and experimentally produced the adult in the nasal passages of several carnivores, *Mustela vulgaris, M. furo, M. foetida, Felis maniculata,* and *Canis vulpes.* They identified the adult as *Orchipedum isostoma* (Rud.).

Orchipedum tracheicola (Fig. 1)

Five specimens were removed by K. E. Goellner from the trachea of a white-winged scoter, *Oidemia fusca deglandi* Bonaparte, collected Oc-

tober 8, 1935, by J. Van Tyne and M. Trautman at the mouth of the Detroit River, Wayne Co., Michigan. One specimen was immature and in the absence of eggs certain structural features were clearly visible. The characteristic excretory and reproductive systems are shown in the immature form (Fig. 2).

Although I have identified these worms as *O. tracheicola,* certain points of difference may exclude them from that species. The most significant difference is the presence of an esophagus in my specimens, whereas Braun described the esophagus as missing. However, his figure shows a protrusion from the pharynx at the corresponding position of the esophagus in the worms here described. The esophagus is a thin-walled structure, usually doubled back dorsad from the pharynx which partially obscures its presence. The specimens on which Braun's description is based had been in the Vienna Museum for more than forty years and probably were in poor condition for staining. Thus the esophagus could easily have been overlooked.

The following measurements are given in millimeters; in each case, the measurements of our American form precede those of the European, and the length precedes the width: body, 6.04×1.28, 7.0×1.6; oral sucker, 0.36×0.42, 0.40×0.48; pharynx, 0.22×0.21, 0.24×0.23; esophagus, 0.17, missing; acetabulum, 0.66×0.66, 0.62×0.83; ovary, 0.34×0.28, 0.50×0.50; eggs, $0.067 - 0.075 \times 0.045 - 0.052$, 0.062×0.050.

It may be noted that the most significant differences are in the size and shape of the ovary and the apparent absence of an esophagus in Braun's description. The size and shape of the ovary has been frequently observed to vary with individual parasites according to the state of development of the worm and the number of eggs present. In each of the four mature parasites the uterus is greatly distended with eggs and the ovary is pressed laterally into an oval form. However, in the immature specimen without eggs, the ovary is circular. The specimen figured and described by Braun as having very few eggs has a circular ovary. Thus there appears a correspondence in shape of ovary among those specimens in which there is no crowding by uterine eggs.

Because of the difference in geographic distribution it might be assumed that this American *Orchipedum* should be distinct from the European *tracheicola.* However, it should be noted that the two hosts of this parasite are to be considered as geographic races only. Although their ranges are no longer contiguous, it is quite possible that many of the parasites common to both subspecies have been successfully perpetuated.

It is peculiar that this species has never been reported since its description by Braun, but it is probably only infrequently that scoters and their closely related species collected in this country or in Europe are submitted to parasitologists for thorough examination.

SUMMARY

A kidney trematode, *Tanaisia pelidnae* n. sp. is described from the red-backed sandpiper, *Pelidna alpina-sakhalina* (Vieillot) with a brief history of the family Eucotylidae and a modification of the generic diagnosis for the genus *Tanaisia*. Trematodes of the genus *Orchipedum* are reported from the trachea of a white-winged scoter, *Oidemia fusca deglandi* Bonaparte. A brief history of the family Orchipedidae and the single genus *Orchipedum* is given with a discussion of the occurrence in North America of *O. tracheicola*, previously assumed to be European in distribution.

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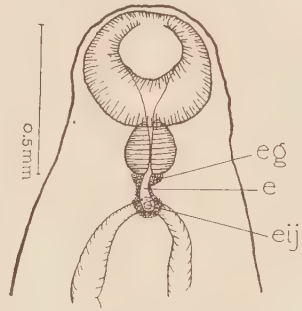
Explanation of Plate

Fig. 1. *Orchipedum tracheicola*, adult ventral aspect. Note ovary on left.

Fig. 2. Immature form. Note ovary on right. (Ovary was dextral in three of the five specimens.) *e*, esophagus; *exb*, excretory bladder; *exc*, excretory canal; *gp*, genital pore; *Mg*, Mehlis gland; *o*, ovary; *ut*, uterus; *vd*, vas deferens. Vitellaria are omitted.

Fig. 3. Details of pharynx, esophagus and esophageal-intestinal junction. *eg*, esophageal glands; *eij*, junction of esophagus and intestine.

Fig. 4. *Tanaisia pelidnae*, ventral aspect. 1 mm scale also applies to figures 1 and 2.



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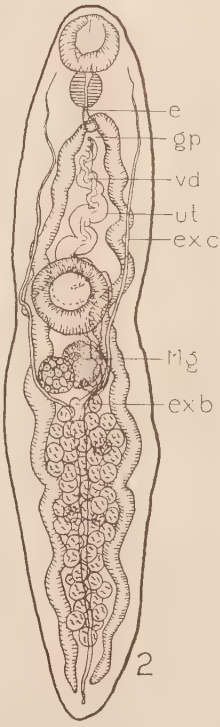

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TICK–HOST ANEMIA: A SECONDARY ANEMIA INDUCED BY *DERMACENTOR ANDERSONI* STILES*

WM. L. JELLISON
Assistant Parasitologist

AND

GLEN M. KOHLS
Assistant Entomologist, United States Public Health Service

The mass rearing of infection-free strains of the Rocky Mountain wood tick, *Dermacentor andersoni* Stiles, is an important part of the routine work of the Rocky Mountain Laboratory. Rabbits are usually used as hosts, and animals heavily infested with adult ticks have frequently died without symptoms or lesions characteristic of any recognized tick-borne infection. For example, a high mortality of this sort was experienced in two lots of 12 rabbits each, infested with infection-free adult ticks on December 9, and December 13, 1932, respectively. Seven of the first lot, and five of the second lot died during the usual 10-day engorgement period of the female ticks. As in other similar instances, subnormal temperatures were noted 24 hours prior to death and autopsy findings suggested anemia. The surviving animals were also anemic as shown by blood tests. The infestations consisting of 80 to 120 adult ticks on each animal, were not appreciably greater than those ordinarily used but were considered close to the maximum that the average rabbit would survive.

Because such losses frequently interfere with the routine rearing of ticks, experiments were undertaken to determine if exsanguination was the only factor involved.

EXPERIMENTAL DATA

The ticks used in the following experiments were of the same stock that had been responsible for the deaths among the two lots of rabbits mentioned above. They were of strains which had been reared under laboratory conditions through several generations during a period of four years and were apparently free of any of the known infectious agents carried by *D. andersoni.*

They were in good condition for feeding, *i.e.,* the females would engorge at the usual rate. In most instances, males and females were used in equal numbers. The test ticks were confined under brass gauze, or tin screw-top capsules, to the clipped bellies of host rabbits.

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* Contribution from the National Institute of Health, Rocky Mountain Laboratory, United States Public Health Service, Hamilton, Montana.

Domestic rabbits of several breeds were used weighing at the beginning of the experiments, from 1.9 to 3.4 kilograms. Infested animals were held in heated laboratory rooms and were given a substantial diet of grain and carrots.

The degree of anemia was determined by erythrocyte counts except in the first and second experiments in which the red cell volume was found by centrifuging samples of citrated blood.

Autopsies were performed on all animals that died, and in some instances transfers of blood or tissue were made to guinea pigs or rabbits.

Experiment 1. This test was designed to determine the approximate minimum number of adult ticks which would produce death in a host rabbit. Citrated blood samples for red cell volume determinations and blood smears were taken from 10 rabbits which were then infested with groups of adult ticks as shown in Table 1. Some were infested with males only, part with females only, and the others with ticks of both sexes. Two control rabbits were kept under identical conditions, but without ticks. Daily temperature records were kept for all rabbits, and as animals became moribund following a critical drop in temperature, citrated blood samples, smears, and blood cultures were again taken. Each rabbit that died was autopsied, and the attached ticks removed, counted, and weighed.

By the twelfth day, all the ticks on the surviving rabbits had completely engorged and detached. At that time blood smears, citrated blood samples, and blood for cultures were taken from each rabbit and these ticks were also removed, counted, and weighed.

A summary of the test data is presented in Table 1.

All 4 rabbits infested with 80 or more female ticks died, and the citrated blood sample taken immediately before death showed a marked reduction in erythrocyte volume. Their average percentage of red cell volume before infestation was 37 and before death 8. Each host remained afebrile throughout the period of tick attachment and fatal cases exhibited subnormal temperatures 24 to 48 hours prior to death.

Blood smears taken just before death from rabbits numbered 4965, 4966, and 4968 and from surviving rabbit number 4969, on the twelfth day after infestation, were submitted to Surgeon R. D. Lillie of the National Institute of Health, who advised as follows: "Study of the blood smears on 'tick-host anemia' discloses an ordinary type of secondary anemia most marked in rabbit 4965 and least in rabbit 4969. This is the exsanguination type of anemia commonly encountered in man or animals after unusual losses of blood."

The effect of the male ticks alone on their host was not so marked. Rabbits 4967 and 4969 survived infestations of 160 and 320 males, re-

spectively, and showed only moderate reductions in erythrocyte volume and slight blood alteration.

The weight of the fed males was insignificant, 320 weighing but 3.2 gm, approximately the same as only 4 fully engorged females.

Autopsies of the rabbits that died showed pale flesh, spleen, and liver and there was usually edema and subdermal hemorrhage at the site of infestation. The latter reaction was due to irritation from tick feeding.

The 2 control rabbits remained normal.

The data indicated that 80 or more female *D. andersoni,* engorging at a normal rate, may kill a host rabbit in 5 to 7 days. There was no suggestion of any other condition than an exsanguination anemia.

TABLE 1.—*To determine the approximate number of adult* Dermacentor andersoni *which will cause death of a host rabbit*

EXPERIMENT 1

Rabbit		Ticks used		No. of females engorging at end of expt.	Weight of recovered ticks in gm	Period of infestation in days	Red cell volume per cent	
No.	Kg wt.	Male	Female				Before infestation	After infestation
4961	2.7	5	5	5	4.0	12	39	38
4962	2.1	10	10	10	7.9	12	37	31
4963	2.2	20	20	20	12.0	12	36	34
4964	2.0	40	40	39	27.0	12	38	24
4965	2.4	80	80	72	29.9	Died 7	38	8*
4966	2.3	160	160	155	26.4	Died 6	38	9*
4967	2.9	160	0	0	1.6	12	37	32
4968	1.9	0	160	154	23.7	Died 7	38	9*
4969	1.9	320	0	0	3.2	12	44	29
4970	2.1	0	320	294	23.0	Died 5	34	7*
4971	2.1	Control		...	...	.....	35	40
4972	2.4	Control		...	...	.....	35	42

* Blood samples taken just prior to host's death.

Experiment 2. This test was designed to determine more closely the number of female ticks constituting a fatal infestation for rabbits. Red cell volume determinations were made on 6 rabbits, which on the following day were infested as shown in Table 2, each with equal numbers of males and females. Red cell volume determinations were again made 7 and 10 days after infestation and red cell counts 7, 10 and 14 days after infestation. The ticks on the surviving animals had completed feeding by the tenth day and were removed.

All rabbits infested with 60 or more female ticks died. Rabbit 4981, infested with 40 females, showed only a slightly reduced red cell volume and count, while 4982, infested with 50 females, showed a considerably greater reduction. It is of interest to note that the red cell count of animal 4981, taken on the fourteenth day, was again practically normal and that 4982 had almost doubled in the 4 days after the ticks were removed. Autopsies of fatal cases were comparable to those in experiment 1.

This test indicated that as low as 60 female *D. andersoni* engorging at a normal rate may kill a host rabbit. Also that in case of nonfatal

infestations, a normal red cell volume is regained rapidly after the cessation of tick feeding.

Experiment 3. In experiments 1 and 2 all the ticks were placed on a host animal simultaneously and, therefore, completed their engorgement on about the same date. This condition is not comparable with the usual rate of infestation by this tick in nature, where a few ticks at a time are picked up over a period of several months. It is obvious that a greater total number of ticks could be fed on a single host by feeding fewer ticks at any one time and extending the period of infestation, provided no infective agent or cumulative effects were present.

Infestations in experiment 3 are more comparable to natural conditions. Tick infesting capsules with screw top lids were applied to a series of 5 rabbits and ticks were added daily to each animal as shown in Table 3. The experiment was continued until the site of infestation under the capsules became so encrusted that it was impossible for additional ticks to attach. As females completed engorgement they were removed, together with an equal number of males. The complete series was maintained for 22 days but 4 of the host animals were observed for a longer period. Red cell counts were taken 9, 13, 17, 22 and 29 days after infesting.

None of the infestations were fatal, and toward the end of the experiment the red cell count in all animals was rising because newly added ticks were unable to attach on the encrusted area under the capsule and the ticks that were already attached were feeding slowly. Nearly twice as many females engorged on rabbit 4991, without seriously inconveniencing the host, as were found fatal in experiment 2.

Although fatal anemia was not produced in any of these hosts it is significant that in general, up to the twenty-second day, the degree of anemia was directly proportional to the number of ticks infested and that the red cell count approached or regained its normal level rapidly as the ticks were removed or the condition of the skin prevented feeding.

These data also suggest that only an exsanguination anemia was present.

Experiment 4. A fourth experiment was planned in order to secure further evidence in regard to the question of whether or not an infectious agent might be concerned. A number of rabbits were each to be infested with nearly a sufficient number of ticks to cause death if continuous feeding were permitted. The ticks from one-third of the host animals were to be removed and weighed on the third day, from another third on the seventh and from the last third on the tenth day, if any of this group still survived. On these same days a red cell count was to be made on each host rabbit. If it should be found that there was an improvement in the condition of each host animal following tick removal,

TABLE 2.—*Futher tests to determine the approximate number of adult* Dermacentor andersoni *which will cause death of a host rabbit*

EXPERIMENT 2

Rabbit		Ticks used		Weight of recovered ticks in gm	Red cell volume per cent			Millions red cells cu. mm		
No.	Kg wt.	Male	Female		Initial	Day 7	Day 10	Day 7	Day 10	Day 14[a]
4981	3.0	40	40	20.8	35	31	31	4.8	4.8	5.2
4982	2.9	50	50	24.6	43	24	24	2.2	2.5	4.7
4983	3.1	60	60	34.3	36	10	Died 8th day	1.3		
4984	2.5	80	80	30.2	42	8	Died 7th day			
4985	2.2	90	90	8.1	40	Died 6th day				
4986	2.0	100	100	12.4	40	Died 6th day				

[a] This is 4 days after removal of ticks.

TABLE 3.—*Data relative to the red cell count of rabbits when a few adult ticks are infested daily over a considerable period*

EXPERIMENT 3

Rabbit		Ticks added daily		Total females used	Total females recovered	Weight of recovered ticks in grams	Millions red cells cu. mm				
							Days after first infesting				
No.	Kg wt.	Male	Female				9	13	17	22	29
4987	2.4	1	1	27	23	13.3	6.2	5.4	4.5	3.5	4.3
4988	2.5	2	2	54	47	18.2	5.3	5.2	4.1	4.7	4.5
4989	2.8	3	3	66	51	25.9	5.3	4.7	5.1	5.7	5.6
4990	2.8	4	4	96	95	48.5	4.7	2.8	3.5	3.4	4.8
4991	3.1	5	5	125	108	49.5	4.2	3.0	3.3	3.6	5.7

as shown by the red cell count and its general condition, this result would strengthen the evidence of the previous experiments against the probability that an infectious condition was concerned.

Nine rabbits were used and each was infested with 150 ticks, 75 of each sex. According to the data of experiments 1 and 2 this number was expected to cause the death of all the rabbits on which the ticks were left for longer than 7 days. However, this did not prove to be true since only one rabbit died, this being one of the third group.

Why more or these rabbits did not die is not evident, since the weight of the ticks removed on the seventh day is equal to the weight of those which caused the death of rabbits carrying an equal or smaller number of females in experiments 1 and 2, except that the rabbits used in this experiment averaged slightly heavier.

Seven of the 9 rabbits showed a decreased red cell count on the third day. Two of the 3 from which the ticks were removed on this day showed a marked rise in the seventh day count and both of these had returned to normal by the tenth day. The third rabbit, however, showed a further decrease in count on the seventh day and a still greater reduction on the ninth day when it died from an undetermined cause but with a red cell count exceeding 5 million.

On the seventh day one rabbit died and the other 5 still carrying ticks showed a further marked reduction of the red cell count. The rate of reduction was definitely greater than that for the first 3 days and for all but one the count was less than 2 million. The 3 rabbits from which the ticks were removed on the seventh day showed an increased red cell count on the tenth day.

Of the group of 3 rabbits only 2 were left. From them the ticks were removed on the tenth day. These two survivors also showed an increased red cell count, although the infesting ticks had been allowed to continue feeding. This was likely because of a lower rate of tick feeding as full engorgement was approached.

These data seem to strengthen the evidence that only an exsanguination anemia is involved. The fact that two of the third group of rabbits recovered suggests that if the rate of exsanguination is not sufficiently rapid to cause death by the seventh or eighth day, then there is a good chance of recovery.

Experiment 5. A fifth experiment was performed in order to secure data by which the degree of anemia produced by tick feeding might be compared with that induced by mechanical exsanguination over a corresponding period. Thirteen rabbits were used and from each one a constant amount of blood was taken daily from the marginal ear veins. The smallest daily amount taken was 2.5 cc; the largest 40 cc. This was continued over a longer period of time than that necessary to cause the

TABLE 4.—*Effect on host of partial and complete engorgement of ticks, each rabbit infested with 75 males and 75 females of* D. andersoni

EXPERIMENT 4

Rabbit		Millions red cells cu. mm				Result	Weight of recovered ticks in grams
No.	Kg wt.	Initial	Days after infesting				
			3	7	10		
5009	2.4	7.2	4.9	6.1	7.0	Recovered	1.8
5010	2.1	7.6	6.7 Ticks removed 3rd day	6.0	5.5*	Died other cause	1.8
5011	2.4	6.4	6.0	6.4	7.3	Recovered	1.8
5006	2.3	7.7	6.4	2.1 Ticks removed 7th day	3.5	Recovered	25.7
5007	2.4	6.1	6.9	1.4	2.0	Recovered	28.6
5008	2.5	6.9	7.7	1.9	2.7	Recovered	32.6
5012	2.4	7.3	6.5	Died 7th day		Died, anemia	31.7
5013	3.2	6.5	4.7	2.0	2.3 Ticks removed 10th day	Recovered	32.5
5014	2.4	6.4	4.6	1.2	2.1	Recovered	35.8

* Rabbit #5010 died of causes other than anemia, the ninth day after being infested: last cell count taken ninth day, prior to death.

death of tick-infested rabbits. Red cell counts were made every fourth day except as otherwise noted in Table 5.

From the first 3 rabbits, 2.50, 3.75 and 5.00 cc, respectively, of blood per day were taken and red cell counts were made through the twelfth day. From the next 4, amounts of 6.25, 7.50, 8.75 and 10.00 cc were drawn and the blood counts made through the twentieth day. All these animals survived and in only 2 which lost 7.50 and 8.75 cc daily did the red cell count drop below 4 million. The largest total volume of blood taken from any of these rabbits was 200 cc.

From the next 3 rabbits the amounts withdrawn daily were 15, 20, and 25 cc, respectively. The third and last count was made on the eleventh day. In these animals the red cell count decreased more rapidly but in no instance fell below 2.3 million. All survived.

From the remaining 3 rabbits the respective amounts of blood taken daily were 30, 35 and 40 cc. The red cell counts dropped abruptly and all died. The 30 cc rabbit died on the tenth day while being bled and the red cell count at this time was 1.6 million; the 35 cc rabbit died on the fifth day under similar conditions and the cell count was 2.4 million; the 40 cc rabbit died on the 4th day with a cell count of 1.7 million.

Experiments 1 and 2 show that the 8 fatalities from heavy tick infestations occurred on the fifth, sixth, seventh and eighth days; that of the rabbits concerned one was infected with 60 females, 2 with 80, 1 with 90, 1 with 100, 2 with 160 and one with 320; that the number of days till death was in inverse ratio to the number of females feeding.

TABLE 5.—*Data relative to the degree of anemia produced by mechanical exsanguination of rabbits, stated amounts of blood being withdrawn daily*

EXPERIMENT 5

Rabbit		cc. of blood withdrawn daily	Total cc withdrawn	Millions red cells cu. mm					
No.	Kg wt.			Initial	Days after initial bleeding				
					4	8	12	16	20
5015	2.5	2.50	50[a]	6.4	7.3	5.6	5.9		
5016	2.5	3.75	75[a]	5.9	5.8	5.1	5.4		
5017	2.6	5.00	100[a]	7.3	5.7	5.3	4.7		
5018	2.9	6.25	125[b]	8.3	6.1	5.5	4.8	4.6	5.3
5019	2.9	7.50	150[b]	7.1	4.4	4.5	3.5	3.6	3.4
5020	3.0	8.75	175[b]	5.8	4.9	3.7	2.9	2.4	4.7
5021	3.4	10.00	200[b]	6.7	6.1	4.9	4.9	5.0	4.0
5022	2.9	Control		7.2	6.3	6.3	6.7	6.2	6.4
							Final		
5023	2.6	15.00	165[c]	7.0	4.4	3.4	2.4		
5024	2.8	20.00	220[c]	6.6	3.7	2.7	3.2		
5025	2.9	25.00	275[c]	6.2	3.6	2.3	2.5		
5026	3.0	30.00	310[d]	6.0	2.3	1.8	1.6	(died 10th day while bleeding)	
5027	3.2	35.00	203[d]	7.0	2.8	(died 5th day while bleeding count 2.4)			
5028	3.5	40.00	180[d]	6.1	1.7	(died 4th day while bleeding)			

[a] Discontinued on 12th day.
[b] Discontinued on 20th day.
[c] Discontinued on 11th day.
[d] Until death.

Experiment 5 indicates that by mechanical exsanguination, 30 cc is the smallest amount of blood that can be withdrawn daily and still effect the early death of rabbits of the size used. In order for the fatalities observed in experiments 1 and 2 to have been entirely due to exsanguination by ticks the amount of blood withdrawn per day by these parasites would presumably have to equal 30 cc or more per rabbit.

Experiment 6. This experiment was planned to determine whether or not the number of female ticks used in experiments 1 and 2 would actually take from their hosts the amounts of blood which experiment 5 indicated as necessary to cause death by exsanguination.

Several attempts were made to determine the actual amount of blood withdrawn by a female tick in engorging. The method first tried was the close confinement of the engorging female in a metal infesting capsule or cloth lined screen capsule on guinea pigs or rabbits so that all the tick feces and the engorged tick might be collected and analyzed for total dry weight and iron content. These trials were unsuccessful due either to failure of the ticks to engorge in close confinement or the loss of tick feces from the capsule.

Finally a plaster cast was made of a rabbit's belly and a depression in the cast served as a capsule. Small holes drilled into the capsule and plugged with cotton permitted ventilation and tick respiration. This cast when well padded with cotton and applied to the host with adhesive tape proved fairly satisfactory for feeding small numbers of ticks.

Host rabbits 3477 and 3478 were infested with 5 and 4 female ticks, respectively, using the above type of capsule. When the ticks completed their engorgement they were removed and weighed and all the feces collected. These materials were analyzed in the Division of Chemistry of the National Institute of Health.

The data of the experiment are given in Table 6. Physical and chemical constants used for rabbit blood (solid matter 183.08 gm per 1000 gm blood; iron 0.43 gm per 1000 gm blood) were as determined by Abderhalden (1898). From the analysis of tick feces and ticks the amount of blood withdrawn by the ticks used in the experiment may be roughly calculated either from the dry weight of the material or probably more exactly from the iron content of the sample.

Through the determination on the basis of total solids, five ticks weighing 2.421 gm after feeding on rabbit 3477, removed a total of 9.669 gm of blood, of which 7.172 gm or its equivalent was passed as tick feces and 2.497 gm retained by the ticks, an average of 1.934 gm per tick or 3.994 gm of blood per gm of engorged tick. On the same basis the four ticks weighing 0.994 gm after engorging on host rabbit 3478 withdrew a total of 9.448 gm of blood, passed 7.935 gm as feces and retained 1.513 gm or its equivalent; an average of 2.362 gm of blood per tick or 9.505 gm of blood per gram of engorged tick.

Somewhat lower but quite comparable data were obtained by calculations based on the iron analysis of the same samples. By this method of calculation from host 3477, 3.642 gm of blood were withdrawn per gram of engorged ticks and from host 3478, 8.322 gm.

The total amount of blood withdrawn by each of the 2 lots of ticks is fairly comparable. However, ticks on host 3478 give a much higher calculated value for the amount of blood withdrawn per gram of engorged ticks, *i.e.,* 8.322 or 9.505 because of the lower final weight of the poorly engorged females. A greater proportion of the blood ingested by them was passed as feces than by the ticks on host 3477.

If we may conservatively assume that the experimental results with host 3477 are more typical of engorgement in this species of tick, then each tick withdraws from 1.7 to 2 gm of blood in engorging or each gm of engorged ticks represents a blood loss by the host of 3.6 to 4 gm.

These determinations are not entirely in agreement with the data of experiments 1, 2 and 5. In experiments 1 and 2 host rabbits died when the weight of the ticks engorging on them approximated 30 gm. According to experiment 6 this would represent a blood loss of 100 to 120 cc, whereas by strictly mechanical exsanguination in experiment 5 rabbits lost from 180 to 200 cc of blood in a comparable length of time before dying.

TABLE 6.—*Determination of amount of blood removed by an engorging tick from its host*

EXPERIMENT 6

	5 ticks weighing 2.421 gm fed on rabbit 3477		4 ticks weighing .994 gm fed in rabbit 3478	
Gms blood determined by:	Iron content	Dry weight	Iron content	Dry weight
In ticks	2.496	2.497	1.498	1.513
In tick feces	6.323	7.172	6.774	7.935
Total	8.819	9.669	8.272	9.448
Per tick	1.764	1.934	2.068	2.362
Per gram of fed ticks	3.642	3.994	8.322	9.505

INFECTIVITY AND CULTURES

Throughout the above experiments there was no evidence secured that the anemia produced was due to an infectious agent although blood and tissue transfers and cultures of the blood were made from part of the host animals showing severe or fatal anemia.

Following the death of rabbits 4966 and 4970 of experiment 1, on the sixth and fifth days of infestations, transfers of suspended spleen tissue were made to normal rabbits by intraperitoneal inoculations. These animals remained well and were released. From rabbit 4984, dead on the seventh day of infestation, 4 cc of citrated blood were inoculated intravenously into rabbit 4959. This animal remained normal and was released. Blood for cultures was taken from several other host

animals showing severe anemia after various periods of infestation. The only instance in which bacterial growth was secured was from blood taken by cardiac puncture from rabbit 4986 several hours after death. This animal showed considerable post-mortem change.

DISCUSSION

Tick-host anemia is apparently a noninfectious condition due to the rapid engorging of female ticks but the possibility is not excluded that the anemia may be contributed to in some degree by a factor or factors other than exsanguination but incidental to tick feeding. Such factors could be a substance from the tick which affects hematopoetic activity or stimulates blood destruction, irritation due to tick feeding, or loss of blood by subdermal hemorrhage at the site of tick attachment.

Critical and even fatal anemias associated with heavy tick infestations have been reported by various writers. Nuttall and Warburton (1908) quoted Lounsbury in reference to *Argas persicus* infestations on chickens as follows: "the victims die entirely from loss of blood and inflammation produced by the excessive parasitism." A rapidly fatal anemia in a horse experimentally infested with "blue ticks," *Boophilus decoloratus* (Koch) is reported by Sir Arnold Theiler (1921). Fenstermacher and Jellison (1933) found severe anemias in moose, *Alces americana,* infested with winter ticks, *Dermacentor albipictus.* Anemia in sheep from *Ixodes ricinus* was observed by McLeod (1933), and in sheep and jack rabbits, *Lepus townsendii,* infested with *Dermacentor andersoni,* by Philip, Jellison and Wilkins (1935). Infestations of fleas, *Ctenocephalides canis,* producing anemia in foxes, have been studied by Law and Kennedy on the Ontario Fur Farm and is the subject of a recent circular (1933).

Severe tick infestations of large game animals, horses and cattle, often accompanied by serious losses, commonly occur. So far no infectious agent has been consistently associated with these losses in the western states and the possibility that an exsanguination anemia is sometimes responsible has not been seriously investigated.

SUMMARY

The writers have repeatedly produced a secondary anemia in rabbits by heavy infestations of ticks, *Dermacentor andersoni.* This condition is noninfectious but is due primarily to exsanguination by the rapidly engorging female ticks and appears comparable to the anemia observed in tick-infested domestic stock and game animals. It is believed, therefore, that tick-host anemia is not only an experimental disease but occurs with some frequency in nature and may be the immediate cause of death in animals.

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A NEW TREMATODE, *ALLASSOGONOPORUS MARGINALIS*, FROM THE MUSKRAT*

LOUIS OLIVIER

Forty-five trematodes were collected from the small intestine of a muskrat (*Ondatra zibethica* L.) trapped in the vicinity of Whitehall, Michigan. The specimens differ notably from all described species and cannot be included in any of the existing genera. The species is named *Allassogonoporus marginalis* n. g., n. sp. and is assigned tentatively to the family LECITHODENDRIIDAE.

The first account of trematodes from the muskrat was made by Leidy (1888). He reported two species, one as *Echinostomum echinatum* Zeder and the other, of which he had only two specimens, as *Amphistomum subtriquetrum* Rud. Barker (1915) regarded the latter as *Wardius zibethicus* and listed nine species, seven of which were described briefly by himself and his associates. Price (1931) added four new species and a key. Recently Warwick (1936) tabulated all of the parasites that have been reported from the muskrat in Europe and North America. This list included twenty-one species of trematodes and, of these, *Notocotylus quinquiserialis, Cladorchis subtriquetrus,* and *Fasciola hepatica* have been collected in Europe. It is probable that certain species listed by Warwick, such as *Fasciola hepatica* and *Paragonimus* sp., are only occasional parasites in the muskrat. It is of interest to note that *Ondatra zibethica* L. is a North American species introduced into Europe.

Allassogonoporus n. g.

Generic diagnosis: Family LECITHODENDRIIDAE. Small distomes, slightly longer than broad. Cuticula without spines. Acetabulum near the middle of the ventral surface. Oral sucker sub-terminal. Prepharynx short. Esophagus extending almost to the acetabulum. Intestinal ceca outside the testes, extending in a wide arc to the posterior part of the body. Excretory vesicle sac-like, extending anteriorly and dorsally between the ends of the ceca. Testes large and ovoid, symmetrically arranged in the posterior half of the worm. Seminal vesicle lateral, large, and elongate. No cirrus or cirrus pouch. Ovary oval, lateral, and anterior to the testes at or near the acetabular level. Seminal receptacle small and spherical, between the testes. Laurer's canal present. Uterus long and convoluted, often filling the posterior half of the worm. Vitellaria composed of many small follicles in a broad area anterior to the middle of the acetabulum. Genital atrium marginal, on either side of the body, near the level of the acetabulum. Eggs small, numerous, and not embryonated.

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Allassogonoporus marginalis n. sp.

(Fig. 1)

Specific diagnosis: Genus *Allassogonoporus*. Characteristics of the genus. Mature worms 0.64–0.90 mm long and 0.59–0.80 mm broad. No spines could be found on the specimens. The outline of the worm is asymmetrical in contracted specimens because the genital atrium is lateral at a level slightly posterior to that of the acetabulum. The genital atrium, which is about 15 μ in diameter, may be on either the right or left margin of the worm. The acetabulum is 0.11–0.15 mm in diameter and is located slightly anterior to the middle of the worm. The subterminal oral sucker is 0.09–0.12 mm in diameter. There is a short, thin-walled

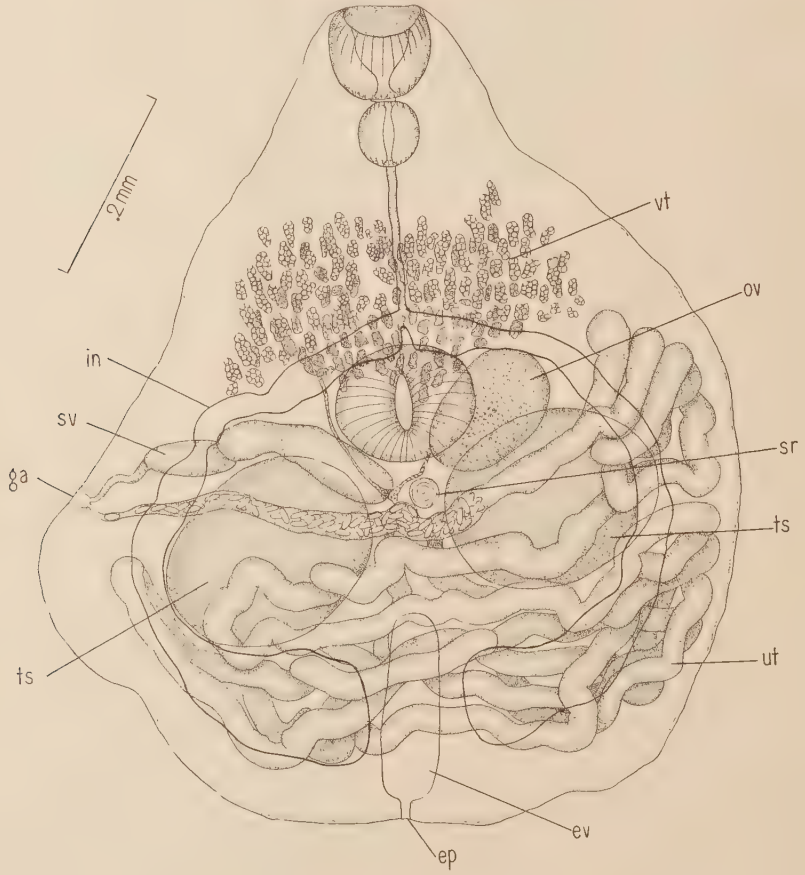

FIG. 1. *Allassogonoporus marginalis*. Ventral view. ep, excretory pore; ev, excretory vesicle; ga, genital atrium; in, intestine; ov, ovary; sr, seminal receptacle; sv, seminal vesicle; ts, testis; ut, uterus; vt, vitellaria.

prepharynx. The pharynx is globular and 0.04–0.07 mm in diameter. The narrow esophagus extends to near the level of the anterior margin of the acetabulum. The intestinal ceca are large and conspicuous, extending in wide arcs to the posterior region where they converge. The excretory pore is terminal and opens into a sac-shaped excretory vesicle which extends anteriorly between the ends of the ceca to

receive two collecting ducts. The testes are ovoid, entire, post-ovarian, and median to the ceca. They measure 0.10–0.20 mm by 0.15–0.24 mm. The vasa efferentia unite to form a short vas deferens leading to the elongate and convoluted seminal vesicle which extends from the region of the female genital complex to the weakly muscular ejaculatory duct. The ovary is lateral and slightly behind the acetabulum on the side of the body opposite the genital atrium. It measures 0.08–0.11 mm by 0.12–0.19 mm. A spherical seminal receptacle, 0.03–0.05 mm in diameter, is present between the testes and ventral to the female genital complex. Laurer's canal opens dorsally. The large uterine loops are in the posterior half of the worm ventral to the testes and ceca. The terminal part of the uterus traverses the worm at the level of the testes to open into the metraterm. The vitellaria consist of many irregularly shaped follicles which lie dorsally in an area extending laterally about three-fourths the distance from the median plane to the margins of the worm and from the second third of the esophagus back to the middle of the acetabulum. The eggs are operculate and not embryonated. Measurements of twenty-one eggs from four specimens ranged from 10 μ by 22 μ to 13 μ by 25 μ.

The worms were fixed in a more or less contracted state and this should be taken into account when evaluating measurements and descriptions. Unless otherwise indicated, measurements given represent the range in size found in ten mounted specimens.

Host: *Ondatra zibethica* L.
Habitat: Small intestine.
Locality: Whitehall, Michigan.
Type specimens: U. S. Nat. Mus. Helm. Coll.

Allassogonoporus does not have a cirrus sac and in this respect it is similar to seven genera in the LECITHODENDRIIDAE: *Lecithodendrium, Anchitrema, Castroia, Ganeo, Lecithoporus, Mesodendrium,* and *Pycnoporus.* These genera either have no cirrus sac or have a pseudo-cirrus sac. *Allassogonoporus* differs from both *Anchitrema* and *Ganeo* in the position of the genital pore, the shape of the excretory bladder, the relation of the ovary to the testes, and the position of the vitellaria. The new genus differs from *Lecithodendrium, Lecithoporus, Mesodendrium,* and *Pycnoporus* in the position of the genital pore, shape of the excretory bladder, and length of the ceca. *Allassogonoporus* differs from *Castroia* in the position of the genital pore, length of the ceca, and shape of the seminal vesicle.

DISCUSSION

Allassogonoporus is similar in certain respects to genera included in the families LECITHODENDRIIDAE and TROGLOTREMATIDAE. Its taxonomic disposition has proved difficult because of its unusual morphology and because of the confusion and uncertainty concerning the limits of these families. The LECITHODENDRIIDAE and TROGLOTREMATIDAE both manifest much morphological diversity and this heterogeneity is reflected in the characterizations of the families. The diagnosis of the family LECITHODENDRIIDAE as given by Mehra (1935) permits so many exceptions and variations of structure and arrangement of organs that the group is not clearly defined. For instance, according to this diagnosis a cirrus pouch may be present, replaced by a pseudo-cirrus pouch, or

absent. The genital pore may be median, sub-median, or sinistral and preacetabular or lateral to the acetabulum. The digestive ceca may be long or short, and the vitellaria may be anterior or posterior to the acetabulum. Taken together, the criteria for the family might admit genera with a wide variety of characteristics. The same criticism might be applied to the family TROGLOTREMATIDAE. According to Wallace (1936) the excretory bladder may be sac-like or Y-shaped, the cirrus pouch may be present or absent, the uterus may be long or short, and the eggs may be large or small. The ovary may be anterior or posterior to the testes, the ceca may be long or short, and in addition, the adults may be located in cyst-like cavities in the tissues of the host or in the intestine.

The lack of morphological unity in these families suggests a lack of genetic unity, but determination of their true status awaits further information. Since the validity of these families is not assured, assignment of the new genus to either of them must necessarily be tentative.

Allassogonoporus is allocated to the family LECITHODENDRIIDAE as outlined by Fuhrmann (1928), Sprehn (1932), and Mehra (1935) because it manifests more agreement with the diagnosis of this family than with that of any other. This genus differs in three respects from Mehra's diagnosis for the family. It has a sac-shaped excretory vesicle while the typical members of the family have either a V-shaped or a Y-shaped vesicle. It differs also in the position of the genital atrium and in the development of the pars prostatica. The allocation of *Allassogonoporus* to the LECITHODENDRIIDAE, although somewhat arbitrary, can be justified even though the genus does not conform to the most recent diagnosis for the family in these three respects.

The form of the excretory bladder has been generally accepted as characteristic for the family LECITHODENDRIIDAE. The shape of this organ appears to be a constant and conservative feature for some of the groups of trematodes, yet, whether its shape is a fundamental family characteristic in all cases is doubtful. McMullen (1935) has shown on the basis of life history studies that in the family PLAGIORCHIDAE the shape of the excretory bladder is not constant and may vary all the way from the long-horned Y-shaped bladder of *Plagiorchis* to the shortened sac-like bladder of *Macroderoides*. Also, in speaking of the untenability of EUMEGACETIDAE Travassos, which had been set up as a separate family on the basis of the shape of the excretory bladder, and which he regarded as a sub-family of the LECITHODENDRIIDAE, Mehra (1935) stated: "Too much importance should not be attached to the shape of the bladder in such systematic considerations." Moreover, *Pleurogenes bicolor* (*Loxogenes bicolor*) has a Y-shaped bladder with very short cornua which is exceptional for the family LECITHODENDRIIDAE as diagnosed. For these reasons it is suggested that the shape of the bladder may not

be constant in the LECITHODENDRIIDAE and as a result *Allassogonoporus* may be included in the family even though it has a sac-like bladder. The position of the genital atrium posterior to the level of the acetabulum should be regarded as a difference of generic rather than family importance. *Allassogonoporus* has a poorly developed pars prostatica but should not be excluded from the family on this account. Fuhrmann and Sprehn do not consider the pars prostatica in their diagnoses of the family.

It seems inadvisable at this time to allocate *Allassogonoporus* to a sub-family of the LECITHODENDRIIDAE. Mehra (1935) set up four new sub-families to supplement the two erected previously by Looss. *Allassogonoporus* will not fit into any of these sub-families and a new one would serve no useful purpose.

In the older literature, trematodes were classified almost exclusively on the basis of adult morphological characteristics, but these criteria alone have proved inadequate as a method for relating the forms. In the last decade workers have come to recognize the significance of the larval stages and life histories as criteria of relationships upon which any natural system of classification must be based. Stunkard (1937) recently stated: "All members of a natural family follow a similar course of development and it has become clearly evident that types of life cycles are closely correlated with phylogenetic and systematic relations. The life cycles of animals, and especially parasitic ones, provide the best evidence of their genetic relations and systematic position." In the LECITHODENDRIIDAE the life cycles of *Lecithodendrium chilostomum* and *Mosesia chordeilesia* have been studied by Brown (1935) and McMullen (1936) respectively. In the family TROGLOTREMATIDAE life histories have been described for *Nanophyetus salmincola,* by Simms, Donham, Shaw, and McCapes (1931) ; *Paragonimus,* by Ameel (1932) ; and *Sellacotyle mustelae,* by Wallace (1935). The taxonomic affinities of *Allassogonoporus marginalis* may be more clearly established when its life history is known. Further life history studies are needed to determine the status of the families LECITHODENDRIIDAE and TROGLOTREMATIDAE.

SUMMARY

Allassogonoporus marginalis is described as a new genus and species of trematodes from the muskrat, *Ondatra zibethica* L. The systematic affinities of this species are discussed and it is placed tentatively in the family LECITHODENDRIIDAE. It is noted that the families LECITHODENDRIIDAE and TROGLOTREMATIDAE are not homogeneous and may not be natural groups. Life history studies are needed to determine the validity of these families and to establish the taxonomic affinities of *Allassogonoporus marginalis.*

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MICROFILARIAL PERIODICITY IN THE CROW

DONALD C. BOUGHTON, ELON E. BYRD AND HORACE O. LUND
Department of Zoology, University of Georgia, Athens, Ga.

Despite considerable investigation since Manson's (1882) original suggestions regarding the mechanism of the nocturnal periodicity of the microfilariae of *Wuchereria bancrofti,* a complete and satisfactory explanation of this phenomenon has not been forthcoming. Because of the sharpness with which periods of activity and rest are differentiated in the crow (*Corvus brachyrhynchos brachyrhynchos*) and the ease with which the behavior of its microfilaria can be modified, the authors believe that this animal and its natural filarial parasite (to be discussed in a subsequent paper) are a more desirable host-parasite combination for the study of microfilarial periodicity than has been employed heretofore. We present here a method of experimentation and some preliminary findings, with special reference to modification of normal periodicity.

Microfilarial counts were made as follows: approximately 0.2 cc of blood was flushed directly from a needle puncture of the shank into a calibrated centrifuge tube containing 14.5 cc of 2 per cent sodium citrate; the tube was shaken and then centrifuged for 10 minutes at 975 r.p.m.; sufficient supernatant fluid was withdrawn to bring the dilution of whole blood to 1 part in 37.5 (twice the volume of sedimented corpuscles was taken as the volume of whole blood); a 0.075 cc sample, withdrawn in a calibrated pipette from the thoroughly agitated suspension, was spread upon a slide and the embryos, usually still motile, were counted immediately under the low power of a compound microscope. Each embryo seen represented 500 per cc of blood. In our experience duplicate counts have no appreciable advantage over single counts. When no microfilariae were found in a 0.075 cc sample, the number per cc was recorded as < 0.5 thousand.

Microfilarial numbers determined for various hours throughout the twenty-four on 4 normal and on 4 experimentally reversed birds are given in Table 1. The hosts kept under "normal" conditions received light and food from 7 to 19 o'clock (i.e., 7:00 AM to 7:00 PM) each day; those under "reversed" conditions, from 19 o'clock of one day to 7 o'clock the next. In these cases only 4 blood samples were drawn, at 6 to 8-hour intervals, during a given 24-hour period; the entire observation on each bird extended over a 3-day period. Such a procedure does not disturb the birds unduly. Three successive appearances of microfilariae, therefore, are actually represented in the figures tabled for each host. For the

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TABLE 1.—*Microfilariae (in thousands per cc of blood) for designated hours of the day in crows under normal and reversed conditions. These figures were obtained from single samples drawn at 6 to 8-hour intervals over a 3-day period*

Crow No.	Hours of Examination (o'clock)								
	24–1	2–3	4–5	6–7	8–9	10–11	14–15	18–19	20–21
	Light 7 to 19 o'clock								
2	6.2	..	5.5	5.8	< 0.5	1.5	0.8	2.5	16.2 41.3* 11.2
8	6.5	0.5	..	7.0	< 0.5	0.5	< 0.5	1.0	11.0* 6.0
11	7.4	..	1.5	7.5	< 0.5	< 0.5	< 0.5	0.6	5.8* 14.0
15	10.0	5.5	..	6.8	0.5	< 0.5	< 0.5	1.0	17.5*
	Light 19 to 7 o'clock								
2	1.4	0.5	1.6	1.3	10.4	14.4	9.1	10.8	1.3
8	< 0.5	..	< 0.5	2.0	32.5 59.0*	48.5	66.0	18.5	0.5
13	< 0.5	..	< 0.5	< 0.5	7.0 6.0*	1.7	4.0	1.5	< 0.5
18	< 0.5	..	< 0.5	3.5	26.5 16.0*	15.0	20.0	24.0	< 0.5

* Determination from a second sample taken 24 hours later.

hours in which a sharp rise was anticipated, two samples were taken on successive days.

With the schedules employed in our work, both normal and reversed periodicities are characterized by: (a) a sharp rise in microfilarial count within the first hour of the host's rest period; (b) a high count maintained irregularly throughout the rest period; (c) a sharp drop within the first hour of the activity period; and (d) a low count throughout the activity period. In some of the low periods no microfilariae were seen in the sample, which, at the dilution employed, indicated that less than 0.5 thousand per cc were actually present in the peripheral blood.

An interval of two days under reversed conditions is sufficient to establish a complete reversal of microfilarial periodicity in the crow. Such sharp reversals were not obtained by Mackenzie (1882) and Low and Manson-Bahr (1934) with *W. bancrofti* in man, nor by Hinman (1936) with *Dirofilaria immitis* in the dog. This may be due to the fact that reversal of the diurnal habits is more difficult in large mammals than it is in small birds. For studies in coccidian periodicity, one of us (Boughton, 1933) has already pointed out that passerine birds are especially favorable hosts, since their periods of rest and activity are more sharply defined than those of certain other animals.

The association of host rest with the appearance and of host activity with the disappearance of this avian microfilaria suggests a causal relationship. This apparently obvious association has been emphasized repeatedly for human and canine microfilarial periodicities by various

writers. The fact of reversal in the crow is, in itself, indicative to some extent of such a relationship. However, the results of several of our experimental modifications of host activity indicate that certain specific alterations in the latter have specific effects upon the number of microfilariae in the peripheral blood. In three such experiments, birds on the normal 7 to 19 o'clock light schedule were subjected to a given change from their normal routine. Counts were made immediately before, during, and subsequent to the experimental shift. In each case, the observations extended from 2 o'clock to 16 or 20 o'clock of the same day. A typical result (one bird) from each experiment is given in Figure 1, in which, for convenience of comparison, the counts are plotted as percentages of the highest count obtained during the period of observation. The bottom panel (D) illustrates the normal periodicity (crow no. 2, Table 1) plotted in the same manner, the observations in this case extending over a 3-day period.

The results may be summarized as follows: When the rest period is begun 4 hours ahead of schedule (by placing the bird in darkness at 15 instead of at 19 o'clock), the microfilarial count rises sharply by 18 o'clock of the same day (Fig. 1, A), at which time under normal conditions the low daytime level would still persist. When the rest period is cut short 4 hours early (by providing the birds with light and food at 3 instead of at 7 o'clock), the count is reduced to daytime level within 2 hours (Fig. 1, B and C), whereas under normal conditions it would remain relatively high until after 7 o'clock. Furthermore, the following sharp rise in microfilarial numbers begins 4 hours ahead of the normal time in birds awakened early; this is true in birds kept awake until 19 o'clock (B) as well as in birds placed in darkness at 15 o'clock (C). There is, however, a slight lag in the former as compared with the latter, which seems to be caused by the continued host activity. We have noted a similar tendency for the early evening high count to be held off temporarily in birds awakened normally at 7 o'clock and given 4 hours of forced activity after 19 o'clock.

It is evident from these results that in the crow the presence or absence of large numbers of microfilariae in the peripheral blood is determined, in part, by the current host behavior (physiological state ?), which has an immediate effect, and to some extent by the antecedent host behavior, the effect of which is evident some 12 hours later. The latter effect is particularly significant. If one supposes the embryos to hide internally during the period of host activity, then, by the act of driving them from the peripheral blood 4 hours early, we have caused these same microfilariae somehow to reappear a corresponding 4 hours ahead of time. If, on the other hand, one supposes the microfilariae to be destroyed between the time of the disappearance of one batch and the appearance of the next,

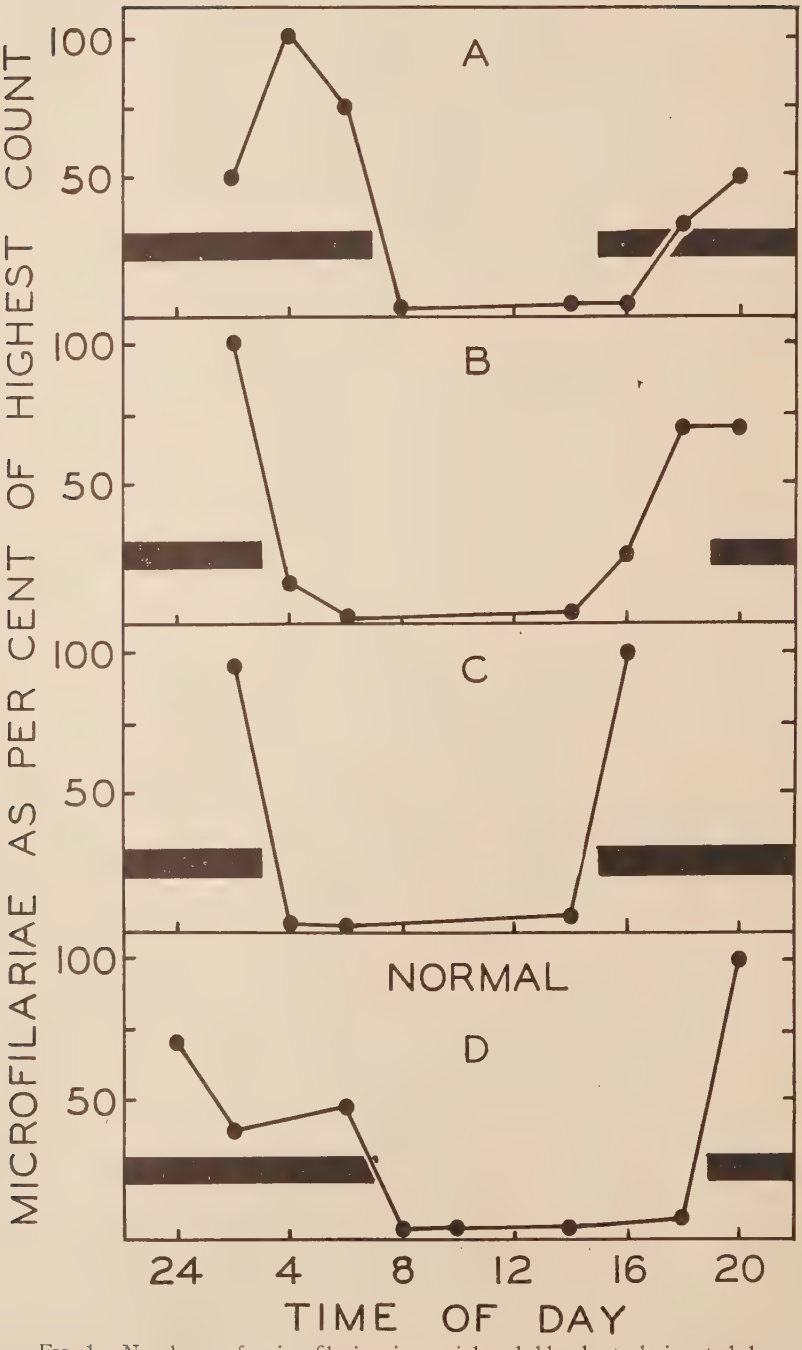

FIG. 1.—Numbers of microfilariae in peripheral blood at designated hours (abscissa) under various conditions of host behavior, plotted for each experiment as percentages of the highest count (ordinate). In the experiments A, B and C, the observations extend in each case over a single 24-hour period; in the Normal (D), over a 3-day period. The solid bars indicate the periods of darkness.

then the early morning host activity has in some manner speeded up the appearance of the microfilariae produced in the interim. According to Lane's (1929) hypothesis of cyclic parturition, this would imply an effect upon the diurnal parturition of the female worms.

In the crow, in which host activity is sharply related to parasite behavior and reversal of nocturnal periodicity is easily accomplished, it should be possible to test out the cyclic parturition hypothesis by comparing the state of development of the embryos in a series of female worms obtained from birds on a normal schedule with that in a series from birds on a reversed schedule. Such a study is now in progress.

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EFFECT OF IRRADIATED ERGOSTEROL ON TRICHINIZED WHITE RATS

WAYNE W. WANTLAND
Department of Zoology, Northwestern University

In a previous publication the writer (1934) reported a marked acceleration of calcification of trichina cysts in white rabbits, effected by oral administration of irradiated ergosterol, together with varying amounts of calcium lactate to provide more calcium for absorption. The results of the above work revealed that irradiated ergosterol in large amounts (80 to 100 drops per day) was decidedly toxic to trichinized rabbits, resulting in death from two to four weeks after beginning treatment. In smaller amounts (30 to 60 drops every other day) irradiated ergosterol produced no deleterious effects, yet markedly accelerated calcification of cysts. Rabbits receiving the lower dosage of ergosterol lived through the fatal period of trichiniasis (5 to 8 weeks) and exhibited neither marked symptoms of the disease nor impairment of muscular movement from calcium deposition. Autopsy of these animals revealed many calcified cysts which upon artificial digestion (0.5 per cent HCl–0.7 grams pepsin) were shown to have contained only dead or disintegrated larvae.

The acceleration of calcification of cysts in the rabbit suggested further experimentation to determine whether accelerated calcium deposition in trichina cysts, resulting from this treatment, was specific in the rabbit or whether it was to be found in other animals infected with *Trichinella spiralis* and subjected to similar treatment. In the experiments reported, the toxicity of the ergosterol preparation was a point of special interest.

FIRST EXPERIMENT

Each of twelve white rats was fed approximately 2500 trichina larvae. Infected rat muscle (diaphragm) was compressed between two glass slides (7.5 cm by 5.0 cm) and an approximate count of larvae made with a compound microscope (low power objective 3×). The amount of infected muscle to be given each animal was placed upon a glass plate (12 cm × 14 cm) and introduced into the cage of the rat to be infected. In each case the animal had not been fed for eight to twelve hours and consequently, quickly consumed all of the infested meat.

Since the deposition of calcium salts in trichina cysts is dependent upon encystment and the presence of at least slight capsule formation, no treatment was administered until the 30th day following infection. Four of the infected animals were then placed on a treatment of 60 drops

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(38 to 40 drops per cc at room temperature—72–75° F as measured by pipette of 1.5 mm bore) of irradiated ergosterol (Mead's Irradiated Viosterol in Oil 10,000 International—also U. S. P.—units of vitamin D per gram) daily, four were given 100 drops daily and four were kept as controls.

Encapsulation was observed to be complete 40 days after infection, but no calcium deposits were noted in the walls of cysts in treated rats on the latest date of autopsy (46 days after infection). These findings differ from the results obtained in previous work of the writer (1934) on trichinized rabbits. The latter animals, when autopsied, revealed slight to marked calcification of cysts five to six weeks after infection, following administration of irradiated ergosterol and calcium lactate per os. The variation in results obtained in the two different rodents may have been due to a somewhat lower calcium intake of the rats. The diet of both rabbits and rats was a balanced one and no rachitic effects were at any time observed.

The dosages of ergosterol used in this experiment were considerably larger respectively, per gram of body weight (15 to 20×), than the amounts given trichinized rabbits. Six of the eight treated animals died from 40 to 46 days following infection. The remaining two, showing marked muscle degeneration and lethargy, were killed on the 40th and 42nd days respectively, following infection. In none of these treated animals was there any evidence of accelerated calcification of trichina cysts.

The rats used in this investigation weighed from 150 to 200 grams when infected. The minimal fatal dose, which generally kills a majority of rats, was about forty larvae per gram of body weight, as shown by McCoy (1931). The infective dose (2,500 larvae per rat) was considered subfatal at the time of infection. It is interesting to note, however, the effect of this number of larvae on three of the four control rats. Two of these three animals died on the 24th day following infection, or during the early muscle phase of the disease. Examination of the diaphragm and other striated muscle revealed numerous larvae in an early encystment stage. A third rat died on the 30th day following infection and fully encapsulated larvae were noted. The fourth control animal lived until the 114th day following infection. Examination of the striated muscle showed a comparatively light infection and larvae were completely encapsulated.

The results of the above experiment are of uncertain meaning. The treated animals at autopsy showed medium to heavy infection of striated muscles and their later deaths, as compared to three of the control animals with corresponding degrees of infection, suggests an increased ability to defend the body mechanism for a few days. This power, however,

was soon lost and the large amounts of ergosterol fed each day seemed to contribute to an early death.

SECOND EXPERIMENT

In a further attempt to effect an accelerated deposit of calcium in trichina cysts in rats, six more animals were infected with approximately 1200 larvae each. This low dosage was given purposely in order to minimize the possibility of death from trichiniasis. Smaller amounts of ergosterol were administered these animals, too, in an effort to reduce the toxicity factor concerned in this substance.

The administration of from 40 to 50 drops of irradiated ergosterol per day to each of three rats, beginning treatment 54 days after infection, resulted in slight to medium calcification of cysts by the 127th day following infection. Earlier biopsies on the 73rd and 113th day revealed no calcification of cysts. Two of the treated rats died 155 days and 157 days, respectively, after infection (see Table 1). Marked polar calcification

TABLE 1

	Ergosterolized rats			Control rats		
	70	71	72	73	74	75
Days after infection ergosterol begun	54	54	54	..	..	..
No. of days ergosterol administered	103	101	106	None	None	None
No. of days infection to autopsy	157 (died)	155 (died)	228	183	195	200
Calcification of cysts	Marked at poles	Marked at poles	None*	None	None	None

* Treatment was discontinued 160 days after infection and the animal lived until killed 228 days after infection. Autopsy revealed resorption of calcium from cysts and 33 per cent of the larvae digested out of various striped muscles exhibited no motility and were non-viable when fed to other rats.

of cysts was noted in both of these animals (see Figs. 1, 2, 3 and 4). The other treated animal (No. 72) was taken off the ergosterol treatment 160 days after infection. This animal was biopsied, for the fourth time, 223 days after infection and the calcium deposits that had been noted earlier had disappeared from all but a few cysts. Some cysts exhibited polar deposits of calcium, but the majority showed no trace of calcium microscopically. It was concluded that the earlier calcium deposits had been resorbed. Approximately one-half of the larvae observed in the material from biopsy of gastrocnemius muscle exhibited signs of disintegration. Twenty-seven larvae were counted in the excised portion, digested out of the cysts by means of artificial gastric juice, and thirteen living (sluggishly active) larvae were recovered. The early calcification of cysts in this animal had apparently led to death and beginning disintegration of many of the encysted larvae. The remainder had withstood the encasement but had been surrounded by the calcium wall for a sufficiently long period to weaken them, as evidenced by only

TABLE 2

	Trichinized rats				Non-trichinized control rats							
	76	77	78	79	80	81	82	83	84	85	86	87
Drops ergosterol daily	20	40	60	80	20	30	40	50	60	70	80	90
Days after infection ergosterol begun	29	29	29	29	..	..	..	..	..	..	..	..
Days from beginning ergosterol to autopsy	159	144 (died)	69 (died)	49 (died)	159	159	145 (died)	98 (died)	86 (died)	64 (died)	51 (died)	42 (died)
Calcification of cysts	Slight	Slight	None	None	..	..	..	..	..	..	..	..

feeble movements even when stimulated by heating to 35° C. Encapsulated larvae were found in the three control animals, autopsied 183 days, 195 days and 200 days after infection, but no degree of calcification was observed in any of the cysts.

THIRD EXPERIMENT

A third experiment was devised in an attempt to determine the relative effects of irradiated ergosterol on trichinized and normal rats. Each of four white rats was infected with approximately 1500 larvae and fed respectively, 20, 40, 60 and 80 drops of ergosterol daily, beginning the 29th day following infection. Eight uninfected rats were fed respectively, 20, 30, 40, 50, 60, 70, 80 and 90 drops of ergosterol daily, beginning the same day as the treatment of infected animals.

The results of this experiment are shown in Table 2. Both normal and infected rats receiving the greater amounts of ergosterol died considerably sooner than those animals receiving lower dosages. Infected animals receiving from 40 to 80 drops of ergosterol per day died a few days before the uninfected rats which had received similar amounts of ergosterol.

DISCUSSION

The results of the above experiments indicate that irradiated ergosterol of the potency used when administered to normal and trichinized rats in amounts varying from 20 to 100 drops daily, eventually gives rise to a toxic effect resulting in death of most of the animals. The rapidity of action and potency of the toxic factor is dependent upon the amount of ergosterol fed. Animals receiving as much as 60 to 100 drops daily generally succumb during the earlier muscle stages of trichiniasis. Irradiated ergosterol fed to trichinized rats results in the same phenomenon of accelerated calcification of cysts as previously observed in trichinized white rabbits treated with irradiated ergosterol and calcium lactate. The rate of calcification was not hastened quite as much in rats as in rabbits. A considerably smaller degree of calcification of cysts than that produced in trichinized rabbits in 38 days following infection and beginning of treatment, required 73 days of treatment in trichinized rats.

At the present time the toxic factor in activated ergosterol is not thoroughly understood nor is there agreement as to its mode of action. Kreitmair and Moll (1928) consider the toxicity produced as due to vitamin D itself through its action on calcium absorption and deposition. Hoyle and Buckland (1929) failed to produce fatal results in rats by feeding large doses of irradiated ergosterol. These authors have emphasized the nonfatal effects and absence of persistent loss of weight. Postmortem examination revealed, however, urinary calculi and slight arterio-sclerosis. In a later paper Hoyle (1930) claims that only when

synthetic diets are used do marked toxic effects appear and a natural diet of bread and milk plus irradiated ergosterol does not give rise to arterial disease and other toxic features. According to Bills and Wirick (1930) when irradiated ergosterol is fed, increased toxicity occurs as calcium is increased in the diet. Again, Harris and Innes (1931) found that as calcium was increased in the diet of rats fed large amounts of irradiated ergosterol, the severity of hypervitaminosis was intensified. At a given level of vitamin D excess an increase of calcium deposition was also noted. These workers report that a hypervitaminosis of a distinctive character may be produced with diets almost devoid of calcium, provided the level of vitamin D excess be raised. Under these latter conditions increased bone resorption occurs but no evidence of calcareous deposition. In the work reported here three trichinized rats were fed a diet sufficient in calcium and remained alive for over three months while receiving daily as much as 10,000,000 times the minimal anti-rachitic dose for rats.

The toxic symptoms produced by large doses of irradiated ergosterol are considered by others to be due to some other factor than vitamin D. The work of Windaus (1931) and Windaus and Luttringhaus (1932) indicates that the toxic effects may be brought about by other substances produced simultaneously with the active vitamin D, during ultraviolet irradiation. These workers suggest that while the ability to destroy anti-rachitic potency by heating, hydrogenation and other methods without altering the toxicity of irradiated ergosterol may indicate two factors concerned, it may be that these procedures affect only one factor, destroying but one of its powers. It is interesting to note in this connection that while the anti-rachitic potency of irradiated ergosterol and related products may be altered or destroyed by heating and other means, leaving the toxicity unaltered, no method has as yet been devised which will bring about the opposite effect, namely, destruction of toxicity with unaltered anti-rachitic potency.

In studying symptoms of viosterol overdosage in human subjects, Reed (1934) found symptoms of toxicity of varying degree in 43 of 300 patients, 7 to 72 years of age, treated with highly concentrated ergosterol (920,000 international units of vitamin D per cc). The dosage ranged from 3,000 to 2,760,000 international units daily, or a maximum of 920 times the normal anti-rachitic dosage. This investigator states that although excessive calcium deposition may be brought about in soft tissues of various animals with relatively low dosages of viosterol, if these animals are allowed to survive until calcium elimination by way of the urine is again normal and losses in weight recovered, no calcium deposits will be found and cellular injuries will have undergone repair. According to the work of Reed there seems to be little need for worry concerning the administration of irradiated viosterol in amounts up to 150,000 international units daily for long periods.

With regard to treatment of trichinized rabbits and rats with activated ergosterol it is the writer's opinion that an amount sufficient to accelerate calcification of cysts, yet produce little or no deleterious effects may be used with benefit to some of these animals. A general dose cannot be stipulated, however, as being efficacious to all, due to considerable variation in these animals in resistance to the parasite, resistance to the toxic factor in the ergosterol and in rate of calcium metabolism. On the basis of the experimental work of the writer, best results are obtained in the treatment of trichinized rabbits. Because of the earlier and more marked calcification of cysts brought about by treatment in the rabbit, the effects of the parasite upon the host are minimized and marked improvement is noticed. In the case of trichinized rats a larger amount of ergosterol per gram of body weight is required to cause even slight calcification, which dosage generally proves fatal to the host. Further experimentation is necessary in order to determine (1) optimum doses of ergosterol which may be administered to various animals infected with trichina, (2) nature of toxicity factor in irradiated ergosterol and (3) effect upon trichina larvae of early encasement by acceleration of calcium deposition in cysts.

SUMMARY

1. Trichinized white rats were studied with regard to (1) the effect of different amounts of irradiated ergosterol per os on rate and degree of calcification of cysts, and (2) the reaction of host to toxicity of the ergosterol.

2. Rats infected with approximately 2,500 larvae and treated with 60 and 100 drops of irradiated ergosterol daily, beginning the 30th day after infection, showed no calcium deposits in cysts after 10 to 16 days of treatment. Rats infected with approximately 1,200 larvae and treated with 40 to 50 drops of irradiated ergosterol per day beginning the 54th day after infection, showed slight to medium calcification of cysts 127 days after infection and marked polar calcification of cysts 157 days after infection.

3. The reaction of normal and trichinized rats to the toxic factor in irradiated ergosterol is dependent upon the amount of ergosterol fed. Uninfected rats fed from 40 to 90 drops of ergosterol daily exhibited definite toxic symptoms and died after 42 to 145 days of treatment. The administration of 60 and 100 drops of ergosterol daily to animals infected with approximately 2,500 larvae, resulted in an apparent increase of resistance to the effects of the parasite up to a certain point, after which the combined effects of the large amounts of ergosterol and the parasite resulted in death.

4. Rats fed fewer larvae (1,200) and given smaller amounts of ergosterol (40 to 50 drops per day) were able to resist the effects of the

parasite and the toxicity of the ergosterol for a considerably longer time. Disintegration of some larvae and resorption of calcium from the walls of cysts was observed after discontinuing treatment in one rat which had been previously shown to contain cysts partially calcified.

5. Irradiated ergosterol per os does not accelerate calcification of cysts in trichinized rats to the extent that it does in trichinized rabbits.

6. At the present time the toxicity factor in irradiated ergosterol is not thoroughly understood. Its nature and mode of action as viewed by some investigators is reviewed.

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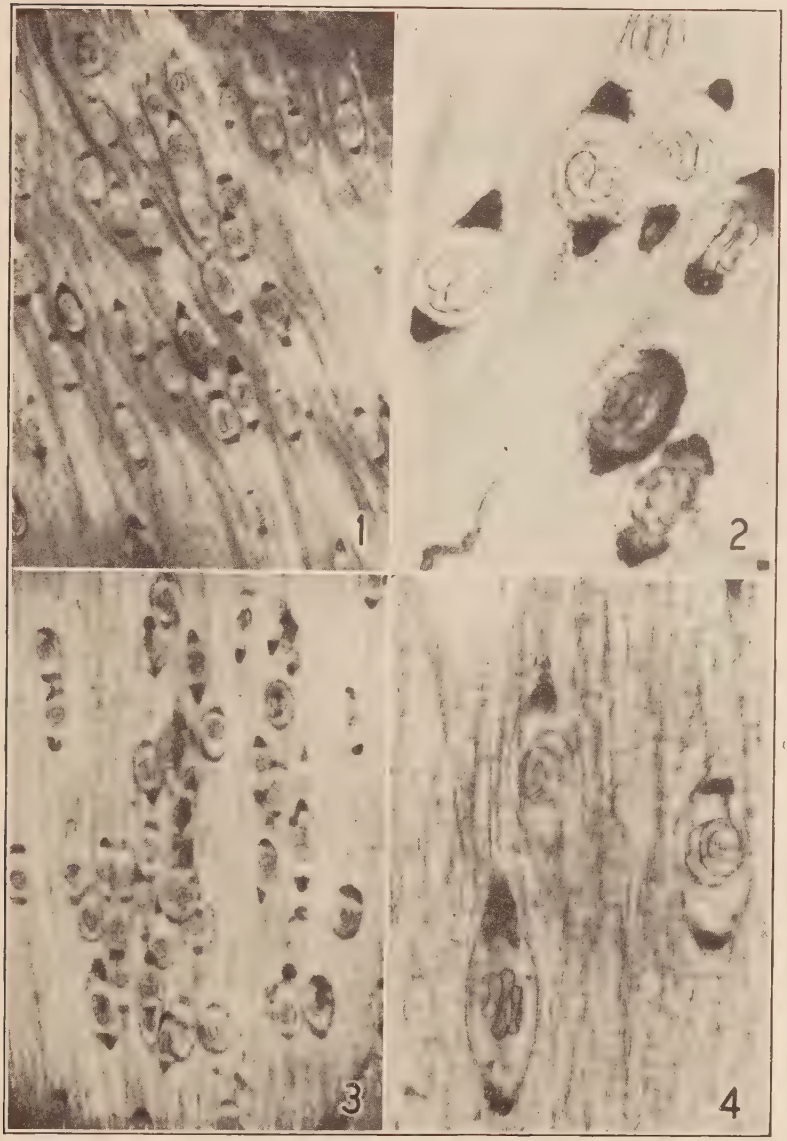
Explanation of Plate

Photomicrographs of different regions of the diaphragms from two white rats (Nos. 70 and 71) infected with *Trichinella spiralis*. Note degree of infection and polar calcification.

Figs. 1, 2. From Rat No. 70, 157 days following infection. Animal received 40 to 50 drops irradiated ergosterol daily beginning the 54th day following infection. Fig. 1, ×32; Fig. 2, ×85.

Figs. 3, 4. From Rat No. 71, 155 days following infection. Animal received 40 to 50 drops irradiated ergosterol daily beginning the 54th day following infection. Fig. 3, ×32; Fig. 4, ×85.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A METHOD FOR THE STERILE CULTURE OF HOUSEFLIES

R. W. GLASER[1]

Department of Animal and Plant Pathology, Rockefeller Institute for Medical Research, Princeton, N. J.

An animal free from living microörganisms and bacteriophage was required to test a theory on the origin of phage. The housefly (*Musca domestica* L.) was chosen for this work because it seemed likely that the entire life cycle of this species could be completed under sterile conditions. The writer, at the present time, wishes to describe a technique which proved satisfactory, and hopes that parasitologists or other workers may find an easily reared sterile host useful. An account of the experiments with phage is reserved for another paper.

a. Sterilization of the Eggs. Housefly egg clusters were obtained from horse manure and placed in a wide-mouthed bottle, capacity 150 cc, with 50 cc of sterile water. The eggs were separated by stirring them for ten minutes with a glass rod rotated by an air turbine motor. The separated eggs were transferred to a 100 cc cylinder and permitted to settle six times in individual 100 cc lots of sterile water. To free the eggs from the remaining debris they were then placed in a solution containing 15 cc of glycerin and 35 cc of sterile water. The fly eggs floated to the surface whereas the debris settled. The eggs at the surface were removed with a pipette and were again sedimented in sterile water six times or until the water was perfectly clear. The eggs were now divided into lots of ½ or 1 cc by volume and sterilized by placing them in 50 cc of a solution containing 5 per cent antiformin in 10 per cent formalin. The eggs were stirred in this mixture for fifteen minutes at 22° C. The antiformin-formalin mixture removed all of the mucus from the surfaces of the eggs, and furthermore inactivated all of the adsorbed bacteriophage. Following this treatment the eggs were immersed in a solution recommended by White[2] for one hour to kill the adhering bacteria. During this procedure the eggs were agitated every ten minutes and were then washed by sedimentation six times in 25 cc of separate volumes of sterile water. When the above directions were carefully followed, the eggs became entirely free of microörganisms and bacteriophages. They were then ready for introduction into a sterile medium which permitted the growth of the maggots.

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[1] The writer wishes to thank Mr. N. A. Coria for his interest and valuable technical assistance.

[2] White, G. F. 1931 Production of sterile maggots for surgical use. J. Parasitol. **18**: 133. Solution is as follows: Mercuric chloride, 0.25 gm; Sodium chloride, 6.50 gm; Hydrochloric acid, 1.25 cc; Ethyl alcohol, 250 cc; Distilled water, 750 cc.

b. Larval Medium. During the preparation of this medium two steps were essential.

1. Swine liver coagulum. To 500 grams of minced fresh swine liver, 1000 cc of distilled water were added. This was heated in a double boiler for 30 minutes until the proteins coagulated. The material was stirred well and the coagulum with its extracts distributed into 250 cc lots, autoclaved and stored in the refrigerator until used.

2. Final preparation of medium. To 75 grams of fine pine sawdust, previously sterilized by dry heat, 250 cc of the swine liver coagulum, 200 cc of distilled water, and 50 grams of Harris Brewers' yeast were added. This mixture was stirred well until the ingredients were distributed so that a uniform soft mash was obtained. Twenty-five grams of mash for each fly culture were packed into a plain 50 cc centrifuge tube (No. 2180 Corning Glass Works). The centrifuge tube was thus filled only to the three-fourths mark, which permitted some expansion of the mash during the subsequent autoclaving.

c. Preparation of Breeding Receptacle. The bottom of a quart milk bottle was covered to a depth of about 3 cm with quartz sand previously washed and sterilized by dry heat. This served later for pupation. A centrifuge tube containing the medium for the maggots was introduced and supported by pressing it into the sand. A vial with food for the adult forms was also inserted and its base lightly pressed into the sand. This vial (Arthur H. Thomas No. 9805, size 70×25 mm) was filled with about 30 cc of a liquid prepared by diluting 100 cc of veal infusion broth with 100 cc of water to which 5 grams of sucrose had been added. The vial was covered with a layer of fine gauze through which the adults could feed. A string was tied to the bottom of the vial, leading up to and through the mouth of the milk bottle. A cotton stopper closed the mouth of the milk bottle and this was capped by a double layer of wrapping paper held in place by a string. The milk bottle with contents was sterilized for 30 minutes at 15 lbs pressure and when cool, the vial containing the adult food was inverted and pulled halfway up the bottle by manipulating the string. In this manner the liquid was held and brought into contact with the gauze covering, giving easy access to the adult flies after their emergence.

d. Inoculation of Breeding Bottle with Eggs. One hundred housefly eggs treated as under *a* were now introduced aseptically by means of a pipette to the surface of the mash in the centrifuge tube. The eggs hatched in about 12 hours at 21–27° C and maggot development proceeded in a normal manner. Under these conditions, with sufficient nutriment for the maggots, the subsequent yield in adults was between 75 and 100 per cent. The mature forms were normal in size and vigor and lived about two weeks. On emergence advantage was taken of their

positive phototropic reactions and they were transferred to a sterile bottle. Here they were etherized and the sexes separated. To produce fresh sterile cultures for experimental use, three males and three females were isolated in each culture bottle. This number, it was later found, was more than enough to produce the required number of maggots and adults. All eggs, maggots, adult flies, their food and environment were continually checked bacteriologically by culture under aerobic and anaerobic conditions.

IN MEMORIAM

George H. F. Nuttall (1862–1937)

In the death on December 16, 1937, of George Henry Falkiner Nuttall, emeritus professor of biology at Cambridge University, biology lost a distinguished investigator and patron, his numerous admirers in America as elsewhere a lovable and generous friend. Throughout his long life he was an ardent pioneer in the field of parasitology. The progress and advancement of that science, and particularly of its entomological aspects, owe much to his vigor, ability and charming personality.

Dr. Nuttall was born on July 5, 1862, in San Francisco of English parentage, his father being Robert Kennedy Nuttall, M.D. He completed his college education at the University of California, and received the M.D. degree in 1884. Later he studied in Germany, England, France and Switzerland, obtaining his Ph.D. degree from Göttingen in 1890 and Sc.D. from Cambridge in 1906. On April 22, 1895, he married Paula von Oertzen, of Kittendorf, Mecklenburg-Scherwin, whose death occurred in 1922.

During his brilliant career Dr. Nuttall occupied many and varied positions. From 1890 to 1893 he was associated with Johns Hopkins University; from 1894 to 1899 he was at the Hygienic Institute of Berlin in the fields of hygiene, bacteriology, and preventive medicine. His period at Cambridge University began in 1899 and in 1906 he was elected to the Quick professorship of biology there, a position which he held continuously till his retirement as emeritus professor in 1933. The Quick professorship of biology was unique in that it was tenable for only three years, when it was thrown open to all who might apply. Dr. Nuttall was successful in being elected each triennium. Over the years Dr. Nuttall and a long list of students contributed greatly to the advancement of biology, especially in the field of parasitology. As the work grew the need for adequate space and equipment became more and more pressing. In May, 1919, Dr. Nuttall prepared a well illustrated brochure describing the needs of the work in parasitology and appealed for funds to construct and equip an "Institute of Parasitological Research." Fortunately the appeal was answered generously and promptly, for on October 23, 1919, Mr. and Mrs. P. A. Molteno contributed the entire amount (£30,000) requested. The Institute, opened on November 28, 1921, is officially known as the "Molteno Institute for Research in Parasitology" and stands as an enduring monument to the memory of Dr. Nuttall and Mr. and Mrs. P. A. Molteno.

During these years of intensive activity he not only contributed numerous scientific articles but found time to take active part in many scientific and philanthropic organizations and in every way to advance the cause of hygiene and preventive medicine, tropical medicine, parasitology and bacteriology. He was a frequent and welcome visitor to the United States, where in 1912 he was the Herter lecturer at Johns Hopkins University, the Harvey lecturer at the New York Academy of Medicine and the Weir Mitchell lecturer at the College of Physicians and Surgeons, Philadelphia. On one of his last visits (1927) he lectured at Johns Hopkins, Chicago and Cornell Universities.

In 1901 in conjunction with Dr. John S. Haldane and Dr. Arthur Newsholme he founded the Journal of Hygiene to serve "as a focus to English-speaking investigators for work in Physics, Chemistry, Physiology, Pathology, Bacteriology, Parasitology, and Epidemiology, in relation to Hygiene and Preventive Medicine." For thirty-seven years he served as its patron and senior editor. In 1908 the field of parasitology had assumed such vital significance to him that he founded "Parasitology." This Journal first appeared under the title "Parasitology, a supplement to the Journal of Hygiene." In the introduction to the first number the editors (G. H. F. N. & A. E. S.) write, "The remarkable development of parasitology in recent years, and the increase in our knowledge of the part played by parasites in human and animal diseases, demand a means of publication in the English language, of original papers dealing with the subject in its widest sense." How well this journal serves the purpose of its founder is well known to students not only in the field of parasitology but to all students of biology. It is of interest to note that the first article of this new journal was on fleas by Dr. K. Jordan and the Hon. N. C. Rothschild and the second by Dr. Aldo Castellani on a liver abscess of a monkey caused by an amoeba, here named *Entamoeba nuttalli*.

Dr. Nuttall was a prolific contributor to scientific journals as well as the author of several important books: "Hygienic Measures in Relation to Infectious Diseases" (1896); "Blood Immunity and Blood Relationship" (1906); "The Bacteriology of Diphtheria" (with collaborators) (1908). In conjunction with Drs. Warburton, Cooper and Robinson he projected a monographic study of the ticks (*Ixodoidea*). The first part appeared in 1908 and several other parts in later years. Though not yet completed the results so far published constitute a monument of excellent, well illustrated, scientific work.

During his lifetime many honors came to him. In 1904 he was elected a fellow of the Royal Society. He was either a member or an honorary member of numerous scientific societies. In America he was foreign honorary member of the American Society of Parasitologists, American Academy of Arts and Sciences, American Academy of Tropical Medicine,

Boston Society of Natural History, Harvey Society, and corresponding member of the American Entomological Society. Among his many honorary degrees were M.A. (Cambridge University, 1900), LL.D. (University of California, 1924), Hon. Dr. (University of Strasbourg, 1927), Hon. D.Sc. (University of South Africa, 1929), Hon. M.D. (Egyptian University, 1928, and University of Liege, 1930). He was Commander, Order of Leopold II, and Commandeur de la Légion d'Honneur.—Robert Matheson, *Cornell University.*

AMERICAN SOCIETY OF PARASITOLOGISTS

Thirteenth Annual Meeting, Indianapolis, Ind., December 28, 29, and 30, 1937*

Minutes of Thirteenth Annual Business Meeting

The Thirteenth Annual Business Meeting of the Society was held at the Claypool Hotel, Indianapolis, Ind., on December 29th, 1937, following the Parasitologists' luncheon. Ninety-eight members of the Society and guests were in attendance. President La Rue called the meeting to order at 1:30 and asked for the reports of officers.

I. Reports of Officers

The report of the Secretary, copy attached, certified as correct by the auditing committee, was presented. It was voted that it be accepted, approved and placed on file.

The report of the Treasurer, copy attached, certified as correct by the auditing committee, was accepted and ordered filed.

The report of the Custodian of the Princeton Secretarial Fund, copy attached, certified as correct, was presented by the Secretary. On motion it was accepted, approved and placed on file.

II. Reports of Committees

The report of the Editorial Committee was presented by W. W. Cort, retiring chairman. A motion was passed expressing the appreciation of the Society for five years of able service on the part of W. W. Cort, Chairman of the Editorial Committee.

The President reported for the Committee on Necrology that three in memoriam articles had been published in the December, 1937, issue of the Journal of Parasitology. The Committee further reported the death of Dr. P. B. A. Powers and subsequently transmitted the following memorial note.

> Philip B. A. Powers, born 14 May, 1907, died 8 December, 1937. A.B., 1929, University of Kansas; A.M., 1930, Rice Institute; Ph.D., 1937, University of Pennsylvania. Instructor at the University of Pennsylvania, Department of Zoology, 1930–1937. His dissertation was on measurements of chromosomes and he had published four papers on the "Ciliates from Sea Urchins."
>
> Doctor Powers was a member of Sigma Xi and in 1936 received the $100 Sigma Xi prize offered by the Pennsylvania Chapter. He was also a member of the American Microscopical Society. In his death the American Society of Parasitologists has lost one of its ablest young members and biological science a promising investigator and teacher.

III. Reports of Representatives of the Society

1. The report of the representatives on the Council of the American Association for the Advancement of Science was given by E. C. Faust. Since the major part of the business of Council had not yet been transacted, the report was preliminary in nature. On motion it was accepted.

2. The report of the representatives on the Council of the Union of American Biological Societies was presented by D. H. Wenrich. He spoke principally of the problems confronting Biological Abstracts and on recommendation of Council presented the following resolutions which were adopted by vote of the Society:

RESOLUTION 1. "The Council of the American Society of Parasitologists would like to take action to insure the continuation of Biological Abstracts. How-

* Abstracts of papers presented were published before the meetings in the Journal of Parasitology (**23**: 547–574). A general report of the program appeared in *Science*, Feb. 4, 1938 (**87**: 106).

ever, it does not believe that it can commit the Society without a referendum. Accordingly, an expression of opinion of the members will be obtained as soon as possible."

RESOLUTION 2. "The Council of the American Society of Parasitologists passed a resolution calling for an expression of sentiment of the members of the Society on the proposition that each member having a regular teaching appointment with the rank of instructor or higher, or an equivalent position in a non-teaching organization, and not contributing through some other society, should be asked to pay $2.00 annually for the support of Biological Abstracts. The Council, therefore, desires an answer to the following questions: 1. Are you in favor of such support? 2. Are you now contributing through any other society? If so, are you in favor of continuing your contribution? 3. If a large majority of the membership of the American Society of Parasitologists favors the action proposed and a $2.00 assessment were voted, would you continue your membership in the Society?"

IV. New Business

The President reported a year of progress for the Society, noting an increase in membership and the maintenance of a high standard in the material published in the Journal.

The Secretary presented the Council nominations for officers of the Society for 1938 as follows: President, for one year: F. C. Bishopp; Vice-President, for one year: E. R. Becker; Secretary, for two years: Oliver R. McCoy. The Treasurer, G. F. Otto, continues for one more year in office. Members of Council for four years (to succeed Eloise B. Cram and Wilbur A. Sawyer): E. C. Faust and H. J. Van Cleave. As members of the Editorial Board for four years (to succeed Charles F. Craig, E. C. Faust and Benjamin Schwartz): W. W. Cort, Benjamin Schwartz and D. H. Wenrich. There were no further nominations and on motion, duly seconded, the Secretary was instructed to cast one ballot for the list as presented.

It was voted that the Society meet next year with the American Association for the Advancement of Science at Richmond, Virginia.

A vote of thanks was extended to the Secretary for five years of service in that office. Furthermore, a vote of thanks was tendered to Raymond M. Cable, local representative at the Indianapolis program for his services in arranging the details of the demonstration tea and luncheon of the Society.

Meeting adjourned at 2:00 o'clock.

Minutes of the Twenty-sixth Council Meeting

The twenty-sixth meeting of the Council of the American Society of Parasitologists was held at the Claypool Hotel, Indianapolis, Indiana, on the 27th of December, 1937. The meeting was called to order at 2:00 P. M. by President George R. La Rue. Those in attendance included the President, J. E. Ackert, W. W. Cort, E. C. O'Roke, G. F. Otto, W. A. Riley, H. W Stunkard and D. H. Wenrich. Letters from absent members of Council and former presidents were read by the Secretary.

I. Reports of Officers

Report of the Secretary was presented and accepted, subject to audit. It was the opinion of members of Council that the Secretary's reports of the Council meeting and annual meeting of the Society should be circularized to members of Council before they are submitted to the Editor of the Journal for publication. Although this procedure has prevented their inclusion in the first number of the following year, it seemed that there was no haste in publishing the record and that accuracy was insured by allowing every member of Council an opportunity to read the reports before publication.

The report of the Treasurer was presented and accepted, subject to audit. The report of the Treasurer included, also, an estimate of income and expenses for the year 1938 and this estimate was approved in principle by Council.

Because of the absence of Norman R. Stoll, the report of the Custodian of the Princeton Secretarial Fund was presented by the Secretary. This report was accepted, subject to audit.

The President appointed E. C. O'Roke and D. H. Wenrich as an auditing committee to examine the reports of the Secretary, Treasurer and Custodian of the Princeton Fund.

II. Reports of Committees

The report of the Chairman of the Editorial Committee was presented by W. W. Cort. It was accepted and a vote of thanks was extended to the retiring Editor for his service in that office.

The report of the Committee on Necrology was presented by W. W. Cort. Since obituary notices for F. W. O'Connor, D. F. Sinitsin and G. F. White were published in the current number of the Journal, the Chairman reported for record the more recent deaths of H. B. Fantham, Philip B. A. Powers and George H. F. Nuttall. It was suggested that a memoriam note for Professor Nuttall, honorary foreign member of the Society, be prepared for publication in the Journal.

There was no report from the Committee on Terminology.

III. Reports of Representatives of the Society

The report of the representatives on the Council of the Union of American Biological Societies was presented by D. H. Wenrich. He stated that Biological Abstracts is in a precarious condition and that unless additional financial support can be secured the Abstracts will be discontinued. He presented a request from the committee in charge of Abstracts that the Society contribute $2.00 per member for the maintenance of the Abstracts. After much discussion the Council voted two resolutions to be presented to the Society. (These resolutions appear above in the report of the annual meeting.)

The Council passed the following additional resolution: "Whereas a great majority of productive biologists in the United States are employed by educational or other similar institutions, and the amount and quality of their research output are largely dependent upon the availability of pertinent literature, support for Biological Abstracts should come primarily from the institutions by which they are employed. In view of the fact that a number of biological societies publish their own journal on income derived in large part from dues, and that many of the members are younger men and women, it would be a hardship for such members to make personal contributions to Biological Abstracts. It appears, therefore, that the subsidy program proposed by the committee on arrangements of Biological Abstracts is more logical than the one calling for contributions from individual biologists."

It was voted that the first two resolutions be submitted to all members of the American Society of Parasitologists and that the third resolution be transmitted to the Council of the American Association for the Advancement of Science and to the Council of the Union of American Biological Societies, the circularization of membership to be made by the Secretary of the Society and the report to the Councils of the A.A.A.S. and U.A.B.S. by the Society representative, D. H. Wenrich.

There was no report from the representatives of the Society on the Council of the American Association for the Advancement of Science.

IV. New Business

During the past year for the first time and following the merger of the offices of the Journal Treasurer and Treasurer of the Society, the Secretary called attention to an earlier provision by Council that the Treasurer should be bonded. Accordingly, arrangements were completed whereby G. F. Otto executed a bond to the amount of $2000 with the Fidelity and Deposit Company of Maryland, Baltimore. This action was referred to Council for an opinion and it

was voted that the $2000 Bond, at a cost of $5.00, is adequate protection for the Society and that this arrangement be continued for the immediate future at least.

Following a suggestion of the Treasurer, Council voted to transfer the membership of H. B. Fantham to Mrs. Annie Porter Fantham, without a formal application on her part in view of her interest and long association with her husband in parasitological work. The recommendation was unanimously approved. The names of seventeen applicants were presented to the Council for election as members of the Society. The Treasurer reported that two of these applicants, C. H. Yeager and E. E. Wehr had been dropped from the Society rolls for non-payment of dues. They had sent to him dues for 1938 with request that they be reinstated without the payment of back dues. He had taken the position that reinstatement by the Treasurer was possible only in case delinquent members paid arrears in full and that otherwise the applicants must apply for election as new members. He asked for statement by Council concerning the correctness of this action and was assured that his judgment agreed with past practice in the Society. The list of candidates was elected to membership with the provision that the election of Lloyd E. Rozeboom be dated as of Jan. 1, 1937, in order that he might receive Volume 23 of the Journal.

The Council then voted stipends of $50 each to the Secretary and Treasurer toward expenses of attending the annual meeting of the Society and $100 to the Secretary for office expenses during the year 1937.

The Executive Committee of the Third International Congress for Microbiology which is to be held in New York the first week of September, 1939, had requested a contribution from the Society toward the expenses of the Congress. In view of the deficit incurred during the year through the publication of the Journal, Council felt that it would be impossible to contribute to the expenses of the Congress. This opinion was adopted by vote of Council.

Continuing the current practice, $10 was voted to the American Society of Naturalists as a contribution toward the expenses of the Biologists' Smoker held in Indianapolis in connection with the current meeting.

At the Council meeting one year ago, the Secretary stated that he expected to have sabbatical leave during the year 1938–1939 and asked at that time that he should not be considered for re-election. It was the urgent request of Council, however, that he accept re-election for the ensuing term, but with the understanding that in case of sabbatical leave he might feel free to resign at the end of one year. In accordance with this understanding, the Secretary notified the President on October 27th, 1937, that he wished to be relieved of the responsibilities of the office of Secretary at the end of the year and President La Rue appointed Justin Andrews, Elery R. Becker, E. C. Faust, E. C. O'Roke and H. J. Van Cleave as a special committee to canvass the field and recommend a successor to the Secretary. Copies of the correspondence are attached and E. C. O'Roke, Chairman of the Committee, reported to Council that Oliver R. McCoy had been chosen as nominee for secretary and that he had indicated his willingness to serve in case he were elected.

The Council of the Society, acting as a nominating committee, voted to recommend the following slate to the annual meeting of the Society: President, for one year: F. C. Bishopp; Vice-President, for one year: E. R. Becker; Secretary, for two years: Oliver R. McCoy. The Treasurer, G. F. Otto, continues for one more year in office. Members of Council for four years (to succeed Eloise B. Cram and Wilbur A. Sawyer): E. C. Faust and H. J. Van Cleave. As members of the Editorial Board for four years (to succeed Charles F. Craig, E. C. Faust and Benjamin Schwartz): W. W. Cort, Benjamin Schwartz and D. H. Wenrich.

It was voted to recommend to the Society that the next annual meeting be held at Richmond, Virginia, in conjunction with the meeting of the American Association for the Advancement of Science.

It was further voted that the Society authorize Chester A. Herrick of the

University of Wisconsin to act as representative of the Society in formulation of plans for the summer meeting of the A.A.A.S. in 1939 in Milwaukee on the same basis as that followed in recent years, namely, that the representative may arrange a program in the name of the Society, but that no funds will be allotted for this purpose.

The Council voted an expression of thanks to the Secretary for five years service in that office.

Council meeting adjourned at 5:30 and in the evening George R. La Rue tendered the complimentary dinner to members of Council and past presidents of the Society at the Claypool Hotel.

Respectfully submitted,

HORACE W. STUNKARD, *Secretary*